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An investigation of a possible serotonergic modulatory input to the auditory system.

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AN INVESTIGATION OF A POSSIBLE SEROTONERGIC MODULATORY INPUT TO THE AUDITORY SYSTEM

by

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Abstract

AN INVESTIGATION OF A POSSIBLE SEROTONERGIC MODULATORY INPUT TO THE AUDITORY SYSTEM

A review of the literature concerning the functions of serotonergic pathways in the vertebrate central nervous system is presented. This information in conjunction with current perspectives on the neuronal substrates of audition led to the proposal that there may exist a modulatory input to the classical auditory pathways at the level of the cochlear nucleus. It was further postulated that this input is serotonergically mediated. The purpose of this research was to demonstrate such an input. Based on preliminary work it was also felt necessary to evaluate the applicability of parametric statistical methods to this type of data base.

Nine male albino rats were pretreated with p-chlorophenylalanine in order to deplete brain serotonin levels. It was felt that such a pretreatment would allow the greatest change in brain serotonin content when an experimental injection of DL-5-Hydroxytryptophan (5-HTP) was later administered. All rats were anesthetized with sodium thiopental and subjected to a surgical procedure which allowed visualization of the cochlear nucleus complex. Glass microelectrodes were used to study single

cell responses to an auditory stimulus consisting of a constant amplitude white noise background upon which were superimposed tone bursts tuned to the characteristic frequency of the cell. In each rat, one cell was recorded both before and after the experimental injection. Five rats received the 5-HTP while the other four received only the injection vehicle. In one rat an additional 9 cells were studied; 5 before injection and 4 after injection.

Examination of the nature of the data obtained indicated that the assumptions of homogeneous variance and normality may not be valid for data of this type. Consequently, non-parametric analysis methods were employed. No significant differences due to the experimental manipulations were detected. Consequently no support was obtained for the existence of the hypothesized serotonin input. The possibility that the experimental techniques used were of insufficient sensitivity for the detection of the experimental effect is discussed in detail.

LITERATURE REVIEW AND INTRODUCTION

Serotonin (5-Hydroxytryptamine) is suspected of being a major neural synaptic transmitter substance (Cooper, Bloom, Roth, 1974). Still to be elucidated, though, are the functional roles of the serotonergic neurons in the overall operation of the brain. The current research was undertaken with the intent of demonstrating the activity of a serotonergic mechanism in the mammalian auditory system.

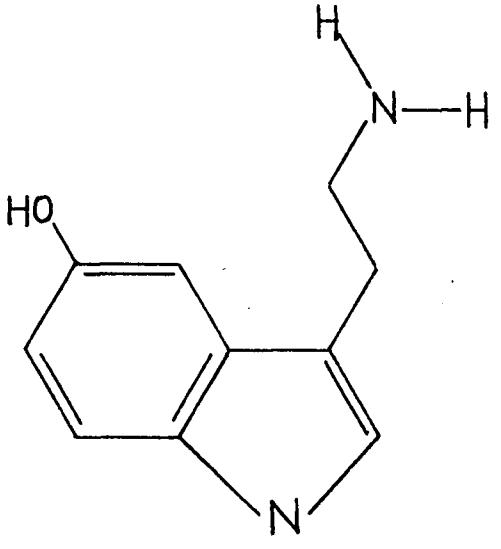
Pharmacology of 5-Hydroxytryptamine (5-HT)

The following discussion is based upon Cooper, Bloom, and Roth (1974). The indolealkyl amine serotonin has the chemical structure as shown in Figure 1 and is distinctively characterized by its double hydrocarbon rings.

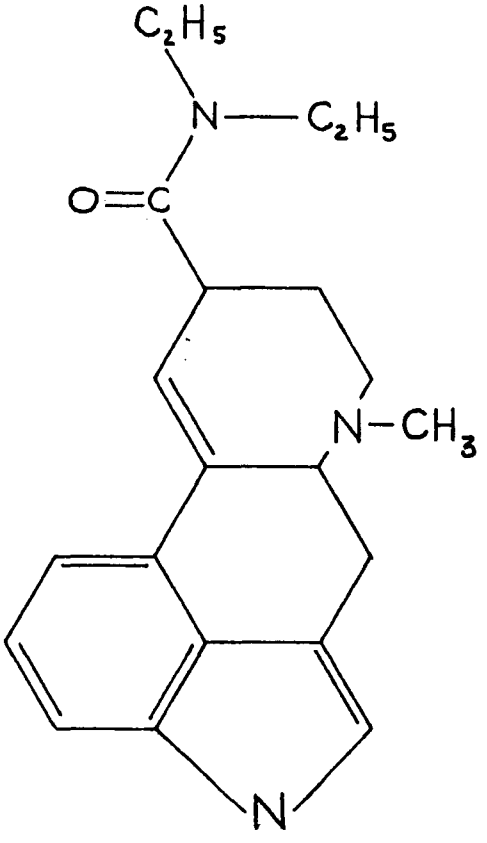
In mammals, most of the body's serotonin content is found peripherally in the enterochromaffin cells of the intestinal tract and as a constituent of blood platelets. Only one or two percent is found in the central nervous system. Although occurring widely in nature and in foodstuffs, 5-HT does not enter the brain readily from the bloodstream. Consequently, it must be synthesized in the brain. The primary dietary substrate for 5-HT production is the amino acid tryptophan. Cellular uptake of tryptophan is facilitated by an active process and the entry sites are competed for by other amino acids such as phenylalanine (the dietary substrate for catechol-

Figure 1

Chemical Structures of 5-HT and LSD



5-HT



LSD

amine synthesis).

5-HT is produced from tryptophan by the following sequence of enzymatic reactions. Through the action of tryptophan hydroxylase, the tryptophan molecule is hydroxylated at the 5 position of the benzene ring to form 5-hydroxytryptophan (5-HTP). This hydroxylation is the rate limiting step in the synthesis of 5-HT (K_m = 3 x 10^{-4} M) although under normal conditions the actual brain concentrations of 5-HT are determined by the availability of tryptophan from the blood and by the availability of the hydroxylase cofactors such as oxygen and pteridine. 5-HTP is almost immediately decarboxylated, thus giving rise to 5-hydroxytryptamine. The enzyme involved in this step is generally known as "aminoacid decarboxylase" and is thought to be identical with the decarboxylase involved in the conversion of dihydroxyphenylalanine to the neurotransmitter dopamine.

The catabolism of 5-HT in the brain is primarily through deamination by the enzyme monoamine oxidase (MAO). (5-HT breakdown in the pineal gland occurs via a different sequence of steps resulting in the formation of the pigment melatonin.) The product of this deamination is further oxidized in the presence of aldehyde dehydrogenase to form the end product 5-hydroxyindoleacetic acid (5-HIAA). This latter substance is eliminated from the body thus forming a constituent of urine. It is important to note that as in the case of the amino acid

decarboxylase, MAO and aldehyde dehydrogenase perform similar functions in the metabolism of the catecholamines (dopamine, norepinephrine, and epinephrine). As a consequence of the nonspecificity of these enzymes, control of the synthesis or breakdown of 5-HT through the manipulation of these enzymes will also have effects on the metabolism of the catecholamine neurotransmitters. Such effects cannot be ignored in the interpretation of pharmacological experiments.

There are several common methods which may be used to manipulate the 5-HT metabolic pathway. The most straightforward method involves control of blood tryptophan levels. Elimination of dietary tryptophan can effectively lower brain 5-HT levels (Wurtman & Fernstrom, 1974). Conversely, an increase in dietary or serum tryptophan can result in increased synthesis of 5-HT (Chase and Murphy, 1973). Such a manipulation may not result in increased brain levels of 5-HT unless accompanied by the administration of an MAO inhibitor such as tranylcypromine (Grahame-Smith, 1971). It is of interest to note that Fernstrom and Wurtman (1973) have also reported that in rats a high carbohydrate diet can increase brain serotonin levels as a result of insulin induced elevations in blood tryptophan. Manipulations of 5-HT levels by dietary control suffer from the nonspecificity of MAO inhibitors and from the possible contamination of experimental effects by alterations in peripheral 5-HT activity.

The direct administration of 5-HTP has been used successfully to increase brain levels of 5-HT. Harvey and Lints (1971) report that interperitoneal injections of 5-HTP can double brain serotonin levels within one half hour after injection. They have also reported rapid induction of normal and supranormal 5-HT levels in rats previously depleted of brain 5-HT stores by lesions of serotonergic neurons in the raphe nuclei. Jouvet (1974) has employed a similar technique to restore 5-HT levels in cats treated with p-chlorophenylalanine (PCPA; see below). Although these methods may also affect peripheral 5-HT levels they have the advantage of allowing rapid alteration of whole brain transmitter concentrations.

Perhaps the most widely used method of altering central serotonin levels is through the administration of p-chlorophenylalanine (PCPA). The depletion of 5-HT by PCPA as first reported by Koe and Weissman (1966) is quite specific to serotonergic systems, but Weissman (1973) points out that PCPA does cause a slight but significant depletion of catecholamines for a few hours after administration. The lowered levels of 5-HT persist much longer, normal values not returning for 7 to 14 days. The specificity of PCPA, or perhaps PCPA byproducts, for 5-HT synthesis is a result of its particular ability to block the action of tryptophan hydroxylase. The inactivation of this enzyme prevents the synthesis of new

5-HT from tryptophan. Consequently, as the endogenous supply of serotonin is used up, the whole brain concentrations fall and are reliably reduced to less than 10% of normal values. As discussed above, this blockade may be conveniently bypassed by the administration of 5-HTP. This compound will be converted to 5-HT by the amino acid decarboxylase which is not affected by PCPA. PCPA thus provides a relatively specific and effective means by which 5-HT levels may be manipulated.

A final consideration in the pharmacology of serotonin concerns the distinction between functionally active and inactive neuronal 5-HT stores. Cooper, Bloom and Roth (1974) cite evidence for the existence of a functionally inactive storage "pool" of 5-HT which complements the active non-storage pool of 5-HT. As Grahame-Smith (1973) points out, this situation complicates the interpretation of experiments concerned with serotonin activity. Most studies of this type are really concerned with serotonin receptor activity but are limited to making inferences about this activity based on concentrations of 5-HT and its metabolites in nervous tissue. Hence it is important to distinguish between changes in tissue concentrations, changes in turnover rates, and changes in receptor activity. Grahame-Smith elaborates by pointing out that assays of 5-HIAA in the urine may provide very poor estimates of 5-HT activity since a significant portion of the 5-HIAA detected could be the product of the metabolism of

functionally inactive 5-HT stores rather than active stores. According to the reports of Cooper et al (1974) cited above, a great deal of serotonin resides in the storage form, thus implying that the functionally active quantities of 5-HT may be relatively small. Given this possibility, it may be questioned whether depletion of brain serotonin levels by any of the known methods results in actual inactivation of the functional serotonin systems. Since no method eliminates 100% of the serotonin of the brain, it is possible that the residual levels are sufficient to maintain at least a significant portion of the normal 5-HT receptor activity.

Neuronal Localization of 5-HT

The studies of Heller, Harvey and Moore (1962) and Harvey, Heller, and Moore (1963) demonstrated that lesions of the raphe nuclei, ventral tegmentum, medial forebrain bundle, and septal area resulted in decreases in brain serotonin levels. These studies provided the first evidence for the discrete localization of 5-HT containing neurons in the central nervous system. However, it was the development of a histochemical fluorescence technique by Hillarp and Falck (for reference and description see Corrodi and Jonsson, 1971) which allowed the extensive localization of 5-HT cell bodies, axons, and nerve terminals

in different brain areas.

Using this method, Dahlström and Fuxe (1964) and Fuxe (1965) presented a detailed description of the distribution of 5-HT terminals and provided relative estimates of the density of terminals in various areas as well as relative comparisons of the size of terminals in those areas. Dahlström, Häggendal, and Atack (1973), having summarized the results of these and other experiments, presented the following observations. The serotonin containing nerve terminals in the central nervous system seem to arise from cell bodies of the raphe nuclei of the brainstem and mesencephalon plus some cells of the reticular formation. These nuclei may be divided into three groups according to the course of axonal projections. Descending bulbospinal axons originate from cells of the n. raphae/pallidus, n. raphae/obscurus, and some of the cells around the pyramidal tracts. Ascending neurons to the tel- and di-encephalon are located mainly in the n. raphae dorsalis and the n. raphae medianus. Innervation of the lower brainstem and cerebellum is through short axon cells situated lateral and dorsal to the pyramidal tract and trapezoid body and through cells of the n. raphae/pontis. As a result of this diffuse projection system, 5-HT containing nerve terminals are found throughout the brain and appear to be present in most parts of the CNS. Furthermore, these studies have shown that 5-HT terminals synapse on nerve cell bodies and

processes which do not fluoresce, presumably indicating connections with non-serotonergic cells.

Accoring to Dahlström et al (1964), the ascending systems in the rat separate into two projection paths. One path travels through the medial forebrain bundle (MFB) and septal area to reach the cingulum and cerebral cortex. A second path proceeds laterally, entering the lateral hypothalamic area and amygdaloid area. Although ample evidence supports the location of 5-HT projections through the MFB in the rat (Harvey et al, 1963; Harvey, Schlosberg, & Yunger, 1975), Parent and Poirier (1969) argue that, in the cat, the 5-HT axons ascend in the cerebral peduncles rather than through the MFB. Recent evidence by Segal (1975) demonstrates the existence of ascending projections to the hippocampus. Studies of monoamine containing cell bodies in the squirrel monkey (Hubbard and DiCarlo, 1973) indicate that the primate serotonin systems are essentially similar to those of the rat.

Physiological and Behavioral Functions of 5-HT Systems

Several authors (Aprison, 1972; Boggan, 1973; Chase & Murphy, 1973; Cooper et al, 1974; Weissman, 1973) have reviewed portions of the literature concerning the involvement of serotonin in behavioral and neurophysiological processes. Weissman classified studies into three groups according to the type of

stimuli evoking the phenomenon under study. These groupings were: social exteroceptive stimuli, non-social exteroceptive stimuli, and no observable stimuli (interoceptive stimuli?)

The literature encompassed under the heading of social exteroceptive stimuli concerned mainly the topics of sexuality and aggressiveness. According to the literature, depletion of 5-HT generally results in enhanced sexuality in both rats and cats. The sexual behavior elicited in such cases "is often aberrant in the direction of an overreaction to an otherwise insufficient or inappropriate sexual object" (Weissman, 1973, pp. 245). Aggressive behavior also seems to be increased by 5-HT depletion and can often be reversed by the administration of 5-HTP. Mouse killing in rats is enhanced and intraspecies aggression in mice, rats and cats is increased by treatment with PCPA.

A wide variety of behavioral effects arising from nonsocial stimuli both externally and internally generated may be mediated, at least in part, by serotonergic mechanisms. The diversity of phenomena that have come under examination defy the formation of general conclusive statements but it is hoped that the following discussion will at least make plausible the few global formulations that have been proposed in the literature.

It has been suggested that 5-HT systems contribute to the habituation of the startle response evoked by loud auditory noises (Conner, Stolk, Barchas, and Levine, 1970; Conner, Stolk, Levine,

1973). Pretreatment with PCPA lowers brain 5-HT levels and significantly increases the number of noise presentations needed to instate habituation. Sheard and Aghajanian (1969) on the other hand were able to produce dishabituation of the startle response by stimulation of the caudal midbrain raphae. This effect could be blocked by administration of PCPA and an additional injection with 5-HTP was able to circumvent the PCPA block and reinstate dishabituation of the response. Conner et al (1973) noted the discrepancy between the effects of PCPA in their studies and those of Sheard and Aghajanian, but were unable to reconcile the differences. To add to the confusion, Harvey and Yunger (1973) reported that lesions of the MFB lowered 5-HT levels but had no effect on noise elicited startle responses. However, MFB lesions would be expected to affect mainly ascending pathways while leaving mesencephalic and descending systems intact. Thus a major portion of the brain serotonin structures would remain functional and might provide the basis for the control of the startle response despite lowering of 5-HT levels in the forebrain.

A considerable quantity of work has concerned the role of serotonin in the modulation of pain sensitivity. In 1971, Harvey and Lints examined the effects of MFB lesions on pain sensitivity as measured by the flinch jump method. MFB lesions produced decreases in the pain threshold. Administration of 5-HTP could counteract this effect. Further studies supported these findings

(Harvey & Yunger, 1973; Harvey, Schlosberg, & Yunger, 1975). Hole, Fuxe & Jonsson (1975), though, reported that selective chemical lesions of the mesencephalic 5-HT pathways did not alter pain sensitivity, although it did reduce locomotor activity. In their 1975 study Harvey et al proposed a distinction between effects on sensitivity as opposed to reactivity to painful stimuli. Based on the observation that MFB lesions affected the jump threshold but did not affect the flinch threshold or startle responses, they concluded that brain 5-HT systems might act to inhibit only the effects of painful stimulation. Of interest in this respect are the reports that exposure to electric shock can affect brain 5-HT activity. Bliss, Ailion, and Zwanziger (1968) have demonstrated that footshock can accelerate 5-HT as well as dopamine (DA) and norepinephrine (NE) synthesis. On the other hand, some investigators (Vermes and Telegdy, 1975) have found that shock results in decreased serotonin activity in the limbic system, particularly in the hypothalamus. Although contradictory in some of the particulars, the above evidence as a whole strongly suggests the involvement of 5-HT mechanisms in the behavioral response to painful stimuli.

Another interesting role of serotonin may be in the performance of learning tasks. Both Brody (1970) and Rake (1973) indicate that depletion of 5-HT by PCPA facilitates performance of passive avoidance tasks. Furthermore, Brody cites evidence that

active avoidance tasks are also facilitated and concludes that the drugged rats were not simply more active than control rats; but rather, PCPA injection interacted sharply with external stimuli to determine the behavior of the animals. However, McFarlain and Bloom (1972) studied U-maze performance after PCPA administration and found no reliable effects.

Interpretation of many of the studies cited and others that are yet to be cited are complicated by the consideration of whether the observable behavioral effects are the result of processes acting on response parameters or whether they are the result of mechanisms involved in the perception of the eliciting stimuli (external and internal). To make a somewhat artificial, though useful, dichotomy, one may ask whether these processes are acting primarily within input systems or within output systems.

As noted earlier, Hole et al (1975) found that lesions of mesencephalic 5-HT neurons reduced locomotor activity. Studies by Modigh (1972) have shown that central administration of 5-HTP produced increases in motor activity while peripherally 5-HTP caused decreases in motor activity. Modigh noted that large doses of 5-HTP can cause displacement of catecholamines by 5-HT in the CNS, yet concluded that this effect did not seem to be the cause of the central motor effects observed. Grahame-Smith (1971) provided corroborating evidence that treatment of rats with tryptophan plus an MAO inhibitor results in hyperactivity. Due to the

fact that tryptophan alone did not produce this result, he suggested that 5-HT can have an excitatory role in the CNS but that normally 5-HT excesses "spill over" onto MAO and are inactivated. An inhibitory role of 5-HT has been suggested by the observation that in Scottish terrier dogs with motor disorders, depletion of 5-HT exacerbates symptoms of excessive muscle tonus (Meyers, Bickson, and Schaub, 1973). 5-HT has also been implicated with the disease of Parkinsonism in humans. This disease also produces symptoms of hypertonicity which can be alleviated by the administration of 5-HTP (Chase and Murphy, 1973).

The results of these studies seem to provide support for an output locus of 5-HT activity. Other studies cited above, particularly those concerning painful stimuli, suggest a locus acting on the input processing mechanisms. To quote Conner et al (1973, p. 333), "Depletion of brain 5-HT clearly results in a greater overall level of responsiveness, particularly when the animal is confronted with a dynamic or abrupt change in the environment. While the available data suggest that this may be due to increased sensitivity in at least several modalities, more systematic data are needed to further substantiate this view."

In light of the highly diffuse projections of 5-HT neurons, it seems entirely possible that serotonin pathways are involved in both input and output circuitry as well as in more central cortical systems. It might be expected from such considerations

that the 5-HT pathways would be involved in the control of general arousal levels and sleep, since these behaviors may themselves have diffuse neural substrates. The research of Takagi, Satoh, Yamatsu, Kimura, and Nakamura (1968) are particularly interesting in this respect. Rabbits were pretreated with tetrabenazine, thus depleting DA, NE, and 5-HT. Stimulating electrodes implanted in the reticular formation, hypothalamus, and thalamus were used to produce EEG arousal. It was then found that administration of 5-HT caused an increase in the level of voltage needed to cause arousal. DA and NE treatment could decrease that voltage level.

General CNS excitability as measured by susceptibility to seizure induction has been studied extensively. Boggan (1973) reviewed the literature on this topic and presented some tentative conclusions. In the majority of studies, drugs which elevate 5-HT levels decreased seizure susceptibility while drugs which depleted 5-HT increased the risk of convulsion. Furthermore, genetic differences in seizure susceptibility were correlated with differences in brain serotonin levels. The study of sound induced seizures in particular have provided a number of research findings which support the above conclusion. The first of these studies (Wada and Ikeda, 1966) found that injection of 5-HTP could strongly inhibit episodic running behavior. In 1970, Schlesinger, Boggan, and Freedman found that, although lowering 5-HT content enhanced seizure induction and increasing 5-HT produced the opposite

effect, the important variable seemed to be the dynamic state of the 5-HT level (i.e. increasing or decreasing), rather than the absolute value of the level. Further interpretational considerations were discussed by Kellogg (1971) who found that mice sensitive to audiogenic seizure (AGS) had serotonin systems which appeared to be inefficient as compared to 5-HT systems of AGS resistant mice. She also concluded that the 5-HT effects might have acted through the midbrain reticular formation by controlling the mediation of the sound stimulus into a reflexive motor response. Boggan (1973) discussed the curious observation that animals previously thought to be non-susceptible to sound induced seizures could be made susceptible by prior exposure to a loud, short "priming" stimulus. In these cases no differences in brain 5-HT content were found between primed and unprimed animals. Tolarmain, Valzelli, and Lehmann (1970), stated that mice that had experienced two full scale seizures did have increased 5-HT turnover.

A further argument for the non-specific locus of 5-HT mediated effects follows from the studies of 5-HT involvement in daily arousal rhythms. Since most animals exhibit obvious cycles of activity and arousal, e.g. waking and sleeping, the case for the involvement of 5-HT in these processes would be supported by the demonstration of daily (circadian) rhythms in 5-HT activity. Such rhythms have been reported (Fernstrom & Wurtman, 1973; Klein,

1974; Morgan, Yudo & McFadin, 1974). Furthermore, these cycles have been correlated with a diurnal variation in pain sensitivity (Harvey et al, 1975). It has also been found (Hutchins & Rogers, 1973) that depletion of brain 5-HT with PCPA can disrupt the circadian rhythm of locomotor activity in mice. The effect of the drug is to produce continuous high level motor behavior. Not only have daily modulations of 5-HT levels been demonstrated, but certain studies (Beckman & Satinoff, 1972; Spafford & Pengelley, 1971) have implicated serotonin in the control of annual hybernation cycles of ground squirrels. This control seems to include modulation of body temperature, a function of 5-HT systems which has been under observation in other laboratories (Meyers, 1973).

Perhaps the most convincing evidence for the role of serotonergic mechanisms in the control of arousal has issued from studies on sleep processes. In 1967, Jouvet presented a paper entitled "The Neurophysiology of the States of Sleep" in which he expounded a theory of sleep control through monoaminergic neural pathways. This theory accounted for the observation that 5-HT depletion by a variety of methods resulted in near permanent wakefulness and could be reversed by 5-HTP (Jouvet, 1974). The essential formulation of the theory posits that 5-HT mechanisms are responsible for the transition from wakefulness to slow wave sleep; a transition which is the result of the inhibition of a catecholamine

arousal (waking) system by serotonergic modulation (Jouvet, 1973). In addition it was proposed that the activity of neurons utilizing norepinephrine is paramount in triggering the onset of paradoxical sleep. Of interest to this hypothesis are the results of a study by Kostowski, Giacalone, Garattini, and Valzelli (1969) in which the midbrain raphae nuclei were electrically stimulated with implanted electrodes. They found that low frequency stimulation induced behavioral calm and sleep while higher frequency stimulation led to alertness and excitation.

The literature thus far discussed has given birth to a variety of generalized statements about the function of the brain 5-HT systems. As mentioned earlier, Conner et al (1973) have concluded that serotonin mechanisms act to regulate sensitivity to stimuli. Weissman (1973, p. 246) makes the following summary statement about the effects of PCPA:

> Animals appear to over-react to painful, electrical, auditory, visual, gustatory, olfactory, and especially social stimuli. Depending on the experimental protocol, this heightened reactivity may be manifested behaviorally as enhanced avoidance, escape or approach behavior, disrupted habituation, heightened motor activity, aberrant hypersexuality, aggressiveness, increased exploration, reduced seizure thresholds, or antagonism of drugs that reduce responsiveness to sensory inputs. The sustained wakefulness of animals treated with PCPA may feasibly be considered part and parcel of this generalized hyperresponsiveness to the environment.

Specifically concerning the role of serotonin in the brain,

Weissman (1973, p. 247) concludes that the evidence may be "interpreted reasonably as suggesting that 5-HT subserves behavioral inhibitory functions as regards responsiveness to exteroceptive stimuli, and that a relative absence of 5-HT, or of 5-HT receptor activity produces hypersensitivity to the environment." Harvey et al (1975) are similarly persuaded in the belief that the 5-HT pathways constitute a general inhibitory system that is not specific to any particular behavioral category. Chase and Murphy (1973) make the conservative statement that the evidence seems to implicate 5-HT in mammalian CNS functioning, but clear understandings of serotonergic mechanisms are not yet available.

In light of the wide anatomical dispersion of 5-HT pathways and the discussion presented above it seems appropriate to say that no particular locus for 5-HT actions may discounted and that a multiple locus of control is likely. Thus the paths of future research may be to elucidate more fully specific mechanisms of portions of the 5-HT systems. To this end, a discussion of the evidence pertaining to the specific nature of serotonin involvement in classical sensory pathways will be undertaken.

5-HT Involvement in Specific Sensory Systems

An examination of the results of Fuxe (1965) reveals that 5-HT nerve terminals are found in a variety of structures encompassed by the classical sensory systems. In the brainstem,

all visceral afferent nuclei studied (N. tractisolitarii, N. commissuralis, N. intercalatus, N. parasolitarius) show a light to moderate 5-HT terminal density. Portions of the general somatic afferent nuclei (N. trigeminus) as well as the dorsal cochlear nucleus possess "scattered" 5-HT boutons. Light to moderate densities are described for the anterior and posterior colliculi. Moving to more central areas, it is found that most of the thalamus including the geniculate bodies exhibit the presence of 5-HT terminals. Paleocortex, involved in olfactory pathways, is lightly scattered with boutons as are most areas of the neocortex. Anatomically speaking, the sensory systems do not lack the presence of serotonergic nerve terminals; thus implying the existence of 5-HT inputs. The possibility that intrinsic 5-HT neurons are located in these areas is not discounted but the general lack of 5-HT cell bodies in areas other than the primary centers (e.g. raphe nuclei) would argue against such an interpretation.

A variety of studies have been published which have examined the effects of iontophoretically applied 5-HT on CNS neurons. These studies have been reviewed (Bloom, Hoffer, Nelson, Sheu, and Siggins, 1973) and have revealed that neurons of several sensory systems are influenced by 5-HT application. In most cases the result was depression of neural activity. Cells of both pyriform and frontal polysensory cortex were influenced by 5-HT. The thalamic ventrobasal N., lateral geniculate N., and medial geniculate N., as well as the olfactory bulb, contain neurons re-

active to serotonin.

Studies of evoked potentials in the visual system have revealed sensory electrophysiological effects of serotonin, of a more molar scale. Rebentische (1968), using rats, found that the amplitude of visual cortical evoked potentials could be significantly decreased by injections of 5-HT. Sabelli and Giardina (1971) obtained similar results in the rabbit, although they also noted that administration of 5-HIAA produced similar effects. A somewhat more complex experiment was reported by Gromova (1970). He found that electrical stimulation of the frontal hypothalamus enhanced photically stimulated potentials. Furthermore, this phenomenon could be influenced by the intravenous injection of 5-HTP. Tebecis (1967a) indicated that neural activity in the lateral geniculate nucleus evoked by visual or optic nerve stimulation could be blocked by doses of 5-HT.

Kawai (1970), using an in vitro preparation, reported that labelled 5-HT (^{3}H-5-HT) was released from slices of guinea pig superior colliculus after electrical stimulation of the optic tract. Also with the aid of an in vitro preparation, Yamamoto (1974) reported that a 5-HT rich perfusate could block single cell discharges elicited by optic tract stimulation and could depress field potentials. Particular mention was made in this case of the apparent normality of synaptic functioning despite the artificial environment.

Fewer studies have been reported implicating 5-HT involvement in other non-visual specific sensory pathways. In a study of the medial geniculate nucleus, Tebecis (1967b) obtained evidence that 5-HT may act as an inhibitory transmitter in that area. Conner et al (1970) found that 5-HT depletion acted to retard habituation of the startle response to auditory stimulation but, as indicated previously, this does not necessarily imply a sensory input system mechanism. Chase and Kopin (1968) have demonstrated the release of labeled serotonin from the olfactory bulb as a response to stimulation with a variety of odors. These investigators specifically argued that this release was not the result of a general arousal reaction, since no such release accompanied arousal induced by footshock or auditory stimulation.

LSD - Its Relation to 5-HT Systems

Further evidence relating to the specific functions of brain serotonin systems has arisen from investigations of the effects of D-Lysergic Acid Diethylamide (LSD 25) on the CNS.

The most salient behavioral aspect of LSD is its psychotomimetic character (Rinkel, DeShon, Hyde & Solomon, 1952). A quote from Key (1965, pp. 30 - 31) is illuminating in this respect:

> Psychotomimetic substances by their definition produce malfunctioning in the perceptual processes. In man not only are visual

> defects symptomatic of LSD 25 administration, but the normal functioning of auditory, somasthetic, and olfactory sensory modalities is also affected. Of the various sensations modified by LSD 25, the emphasis placed on visual disturbances may be a consequence of the fact that, man being predominantly a visual creature, more significance is attached to alterations manifest within this system. In animals, especially cats, change in responsiveness to auditory stimuli appear to play an equally important role . . . Although uncommon, the occurrance of auditory, olfactory, and gustatory illusions and hallucinations suggests that the marked visual disturbances associated with LSD 25 are only a part of wider, more diverse changes in sensory processes.

The possibility that the LSD effects may be mediated through the brain serotonin pathways is suggested by evidence from various sources. A comparison of the molecular structure of LSD and 5-HT reveals a striking similarity as shown in Figure 1 (Berridge & Prince, 1974; Kang & Green, 1970). Chase et al (1973) have noted that, in general, hallucinogenic or psychotomimetic compounds share structural features requisite for antagonistic activity at serotonergic receptors. In a recent study (Bennett & Snyder, 1975) investigators found evidence to indicate that LSD binds directly to the postsynaptic serotonin receptor. Using iontophoretic techniques, Aghajanian, Haigler, and Bloom (1972) were able to demonstrate inhibition of the firing rates of raphae neurons by LSD. The inhibition did not affect the spike amplitudes and was very similar in nature to the effect of the application of 5-HT on the same cells (Aghajanian and Haigler, 1973). In fact it had

been shown that the decrease in firing rates with LSD was accompanied by a fall in 5-HT turnover (Aghajanian, 1972). In the study of Kawai (1970) cited earlier involving release of labeled 5-HT from slices of superior colliculus it was also found that LSD could block this release. In seeming contradiction are the findings of König-Berson, Waser, Langermann, and Lichtensteiger (1970) that psychoactive drugs, particularly LSD, caused increases in brain 5-HT levels if administered systemically. The contradiction may only be apparent if it is realized that blockage of release as reported by Kawai would necessitate the storage of freshly synthesized 5-HT rather than permitting access of the 5-HT to catabolizing enzymes. Thus the net effect would be an increase in the levels of the stored amine.

A series of related studies beginning in 1958 has provided considerable information about the behavioral and electrophysiological correlates of LSD administration. In the first of these studies (Bradley & Key, 1958), it was found that LSD caused behavioral alerting and EEG arousal but that the effect was dependent on particular environmental conditions. This dependence differentiated the effect from that obtained with the stimulant amphetamine. Curiously, it was also found that the drug markedly decreased the thresholds for arousal by auditory stimuli, but did not have any effect on cortical evoked potentials elicited by click stimuli. This latter finding was in contradiction to the

results of Purpura (1956a, 1956b) and Brown (1961) who found that LSD did alter auditory evoked potentials. Bradley et al (1958) suggested that the action of the drug was not on the afferent pathway itself but rather was on the reticular activating system (RAS), sensitizing it to the activity of collaterals to the RAS from afferent pathways. This work was followed by an investigation of the effects of LSD on discrimination and sensory generalization of auditory stimuli in cats (Key, 1961). LSD decreased the rate of extinction of hurdle crossings to auditory stimuli but did not alter the intensity threshold necessary to evoke conditioned behavior. It was then proposed that LSD might increase the meaningfulness or significance level of the sensory stimuli. Four years later Key (1965) published a work in which it was found that LSD produced a significant increase in the mean amplitude of potentials evoked in the dorsal cochlear nucleus if the experiment was conducted with a low ambient noise level. When testing was performed in the relatively noisy open lab there was little effect on amplitude but an increase in the variance of the response was noted. It was then theorized that the drug effects were indirect and were related more to the modulation of the sensory information than to the direct processing mechanisms. Various possible sites of modulation were examined (intra-aural muscles, olivo-cochlear bundle, brainstem reticular formation) but with negative results. It was not until 1970 that these studies were

evaluated in terms of their relationship with serotonergic systems. At that time it was demonstrated that LSD could antagonize the actions of 5-HT on brainstem neurons. Such antagonism could be engendered by both systemic and iontophoretic administration of the LSD. The actions of the drug were summarized by stating that the drug (LSD) appeared to influence, in a highly specific manner, the neurophysiological mechanisms concerned with filtering and integration of sensory information, a disturbance in the balance of which might account for disturbances in perception, and hence produce hallucination. Finally Bradley and Briggs (1974) studied neurons of the reticular formation situated in the pons and medulla. 5-HT iontophoretically applied excited cells, an effect which was antagonized by PCPA, LSD and other LSD-like drugs. They reiterated that these effects provided a plausible explanation for the actions of these psychotomimetic agents in the processing of sensory information.

The research to be presented in this paper was undertaken with the intent of extending the previously described series of experiments by examining the possibility of a 5-HT modulatory input to the auditory pathways, in particular to the cochlear nucleus. Before proceeding to a discussion of this work, it seems advantageous to review previous investigations into the processing mechanisms and neurophysiology of the auditory system with particular emphasis upon the cochlear nucleus.

Anatomical and Electrophysiological Bases of Audition

Figure 2 presents a schematic diagram of the major auditory pathways (after Kay, 1974). The initial transduction of air pressure variations to neural activity is performed by the cochlea. As a result of the work of von Bekesy (1956) it has become evident that considerable analysis of the sensory stimulus may take place in the receptor organ, thus producing a neural output in which different neurons fire in response to a small range of frequencies. Thus in a bundle of such neurons, different tones will cause neural firing in different places in the cross section of the bundle. This particular process has been named the "place theory" of frequency coding. Frequency may also be coded in terms of the temporal relationships between spike discharges synchronized between a number of fibers. Such a process, called the "Volley Principle," allows the coding of sound pressure variations at frequencies much greater than those which could be coded by the limited firing rate of any one cell (Thurlow, 1971).

The cells of the cochlea project via the eighth cranial nerve to the dorsal and ventral cochlear nuclei. The output of the cochlear nuclei then passes to a variety of structures. The dorsal cochlear nucleus (DCN) sends axons mainly to the contralateral nuclei of the lateral lemniscus (NLL) and inferior colliculus (IC). Fibers from the ventral cochlear nucleus (VCN) also project contralaterally to these areas but also send collaterals to the ipsilateral and contralateral superior olivary complex

Figure 2

Diagram of Auditory Pathways

DCN - Dorsal cochlear nucleus

IC - Inferior colliculus

LSO - Lateral, S-shaped segment, superior olivary nucleus

MG - Medial geniculate body

MSO - Medial superior olivary nucleus

NLL - Nuclei of the lateral lemniscus

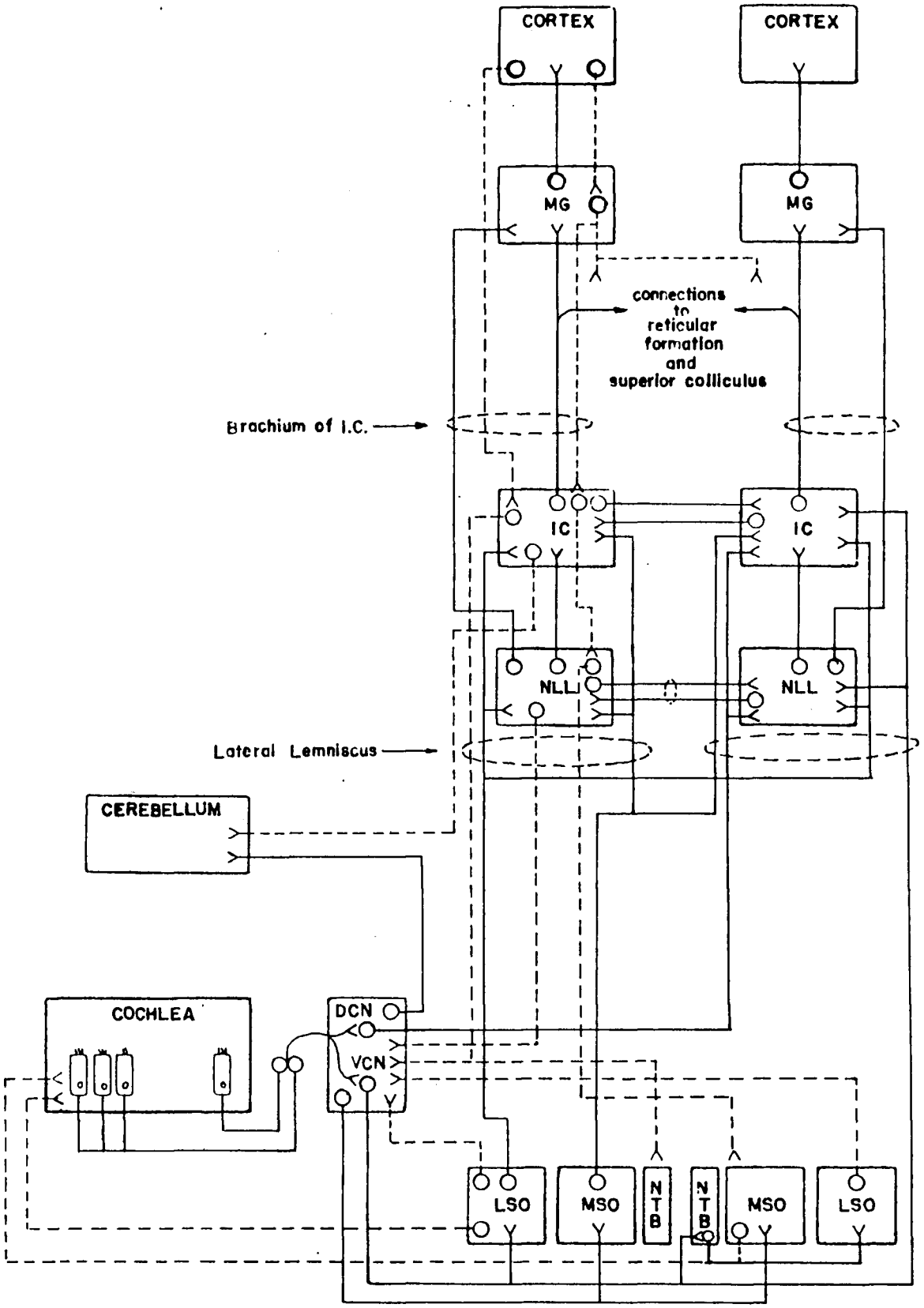
NTB - Nucleus of the trapezoid body

VCN - Ventral cochlear nucleus

Solid lines - afferent pathways

Dashed lines - efferent pathways

Note: For clarity only one cochlear input is shown with its ipsilateral and contralateral pathways.


CORTEX
CORTEX
MG
MG
connections to reticular formation and superior colliculus
Brachium of I.C.
IC
IC
NLL
NLL
Lateral Lemniscus
CEREBELLUM
COCHLEA
DCN
VCN
LSO
MSO
NTB
NTB
MSO
LSO

and nucleus of the trapezoid body. Central projections proceed from the ipsilateral superior olivary complex ascending in the ipsilateral nuclei of the lateral lemniscus to the inferior colliculus. Neurons of the inferior colliculus and nuclei of the lateral lemniscus synapse in the medial geniculate body of the thalamus from which all cortical projections arise. According to Kay (1974) no cells of the cochlear nuclei project higher than the inferior colliculus and no ascending cells of higher nuclei bypass the medial geniculate to reach primary cortical areas. This implies that in the central auditory pathways at least two synapses are encountered between the cochlear nuclei and the cortex. Furthermore, ipsilateral and contralateral pathways are interconnected at every level and projections are made to the reticular formation, especially at the level of the inferior colliculus. This myriad of intercommunications and synaptic levels indicates that processing of the neural information occurs at every level and thus the nature and/or the form of the information arriving at every level may be significantly different.

Not only are the ascending auditory projection routes vastly complex, but they are complemented by a descending efferent system which interacts with the ascending information at several levels. As indicated by the dashed lines in Figure 2, there are efferent connections from the cortex to the medial geniculate and inferior colliculus. Below this level there appear to be two major destinations of the centrifugal systems. One is the cochlea

itself. The efferent input arrives at the cochlea via the crossed olivo-cochlear bundle (OCB) (of Rasmussen). According to Kay (1974), cells of the OCB may receive inputs from all of the auditory cortical areas. The second destination is the cochlear nucleus. Details of these efferent fibers are presented below. Other than anatomical evidence, little is known about centrifugal auditory processes. Their anatomical arrangements place them in a particularly effective relationship with the afferent fibers to control the flow of information to the cortex (gating) or to pre-program the nature of the processing being performed at each level (modulation?)

Since the cochlear nucleus receives essentially all of the output from the cochlea and since it also is the first central processing area which is innervated by descending pathways, it provides an excellent site in which to study central information processing mechanisms. As such it will serve as the anatomical focus of this paper.

Osen (1969) has made a detailed study of the cytoarchitecture of the cochlear nucleus in the cat. He pointed out that his findings agreed well with the findings of Harrison and Feldman (1970) concerning the VCN in the rat, but that since these latter investigators did not examine the DCN it was not certain if the structure of the DCN in the rat is exactly comparable.

Figure 3 is a semi-schematic diagram of a sagittal section through the cochlear nuclei and summarizes the cytoarchitecture as reported by Osen (1969). The complex is conventionally divided into the dorsal nucleus and the ventral nucleus. The latter is further subdivided into the anteroventral and posteroventral divisions. The primary afferents bifurcate into ascending and descending branches. Note that the descending branch first projects caudally and then turns laterally. As indicated, these fibers are arranged tonotopically and agree with the observations of Rose (1960), who found that, in the DNC, cells responding to high frequencies were situated most dorsally while cells situated more ventrally and laterally exhibited a preference for low frequency tones.

Osen (1969) has identified a number of cell types based on morphological differences of which only the major types are shown in Figure 3. Of particular interest is the stratification of the DCN. The central area of this nucleus is populated by cells with distinctively large nuclei, termed giant cells. Circumferentially about this core are arranged a layer of radially oriented pyramidal cells, these being covered superficially by a zone of smaller cells and fine, mostly unmyelinated nerve fibers. The fibers of this latter zone, known as the molecular layer, are primarily oriented parallel to the surface and traverse

Figure 3

Semi-Schematic Diagram of a Sagittal Section
Through the Cochlear Nuclei of the Cat

Key to Figure 3

br.p. - brachium pontis

cap - peripheral cap of small cells

co.f. - cochlear nerve fiber

c.r.d.c.n.- central region of the dorsal cochlear nucleus

gl.c.a. - globular cell area

h. - high tonal frequencies

l. - low tonal frequencies

l.sph.c.a.-large spherical cell area

m.l. - molecular layer

n.vest. - vestibular nerve

o.c.a. - octopus cell area

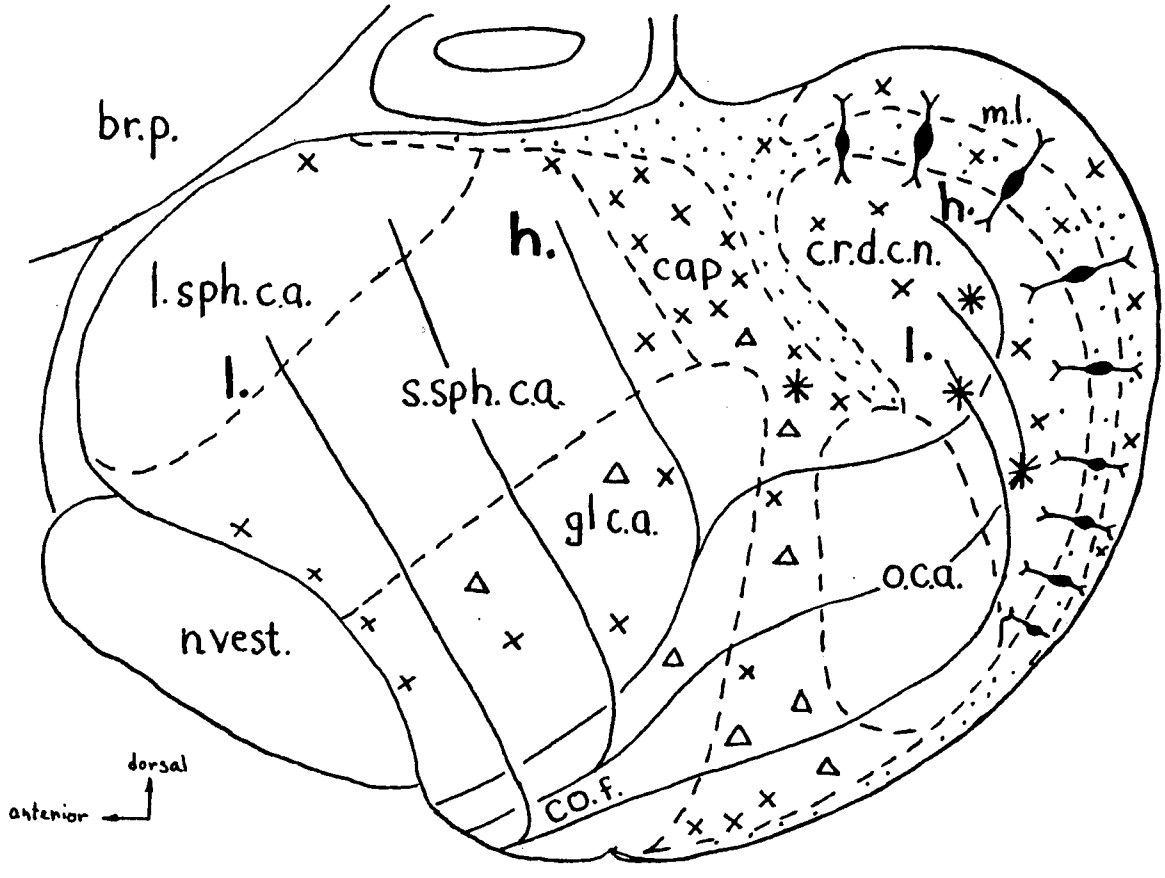
* - giant cells

x - small cells

 - pyramidal cells

· - granular cells

Δ - multipolar cells


br.p.
m.l.
h.
l. sph. c.a.
h.
cap
c.r.d.c.n.
l.
l.
s.sph. c.a.
gl c.a.
o.c.a.
n.vest.
dorsal
anterior
co.f.

in a dorso-ventral orientation. These fibers of the molecular layer are situated at approximately right angles to the cochlear afferents in that vicinity. Both pyramidal and molecular layers are interspersed with numbers of small granular cells. No indication has been made as to which of these cell types are the primary output neurons, though it might be conjectured that the giant cells or large pyramidal cells may be most suited to the metabolic maintenance of a long axon (Shepard, 1974).

A variety of efferent inputs to the cochlear nuclei have been described. Rasmussen (1960) reports that fibers of the olivo-cochlear bundle synapse in the granular cell layer of the VCN. Although the reports are not overly lucid, Rasmussen states that three other efferent paths project into the cochlear nuclei. One input arises partly from the lateral lemniscus and partly from unknown sources. This tract enters the DCN by way of the dorsal acoustic stria which passes over the dorsal surface of the restiform body. A second input originates in the superior olive and ends in the VCN. This pathway is distinct from the collaterals of the OCB and seems to exhibit strong acetylcholinesterase staining reactions (Rasmussen, 1967). (Of interest to this paper are the findings of Comis and Guth, 1974, that a non-cholinergic substance is released from the VCN upon stimulation of the OCB. The substance

is not cholinergic nor are its effects mimicked by NE, or glycine but 5-HT was not tested. Also of interest is the proposal of Pepeau, Garau , and Mulas, 1974, that 5-HT might influence CNS cholinergic mechanisms.) The third set of centrifugal fibers project from the inferior colliculus and nucleus of the lateral lemniscus through the trapezoid body to synapse in the DCN. Osen (1969) also reported efferents similar to this last group and indicated that they penetrate through the giant cell layer to terminate within the same layer as the primary cochlear afferents. Osen (1969), in addition, found fibers, possibly efferent, of unknown origin which synapse in the superficially situated molecular layer. Rasmussen (1966) concluded that, overall, the DCN possesses a preponderance of non-afferent type synapses when compared to the VCN.

The electrophysiology of the cochlear nucleus has been studied in detail. As indicated earlier, a most striking feature of the complex is its tonotopic organization. A similar arrangement has been observed in the cochlear nerve and at higher levels by Kay (1974) and Katsuki, Suni, Uchiyama, and Watanabe (1958). These investigators, as well as Rupert, Moushegian, and Galambos (1963) found that single cells of the nerve responded to a range of tone frequencies, usually firing most rapidly to one particular frequency which they termed the characteristic frequency (CF). For most neurons, the response threshold versus stimulus frequency

relationship shows a more rapid "cut off" at the high frequency end of the response band than at the low end. Roll off rates as high as 200 dB/octave were evidenced. Many variations on this response theme were also encountered. Some cells responded equally well to two different tones. Most units exhibited spontaneous activity in the absence of external stimuli. Katsuki et al (1958) found this to be true for cells as high as the inferior colliculus but that the phenomenon became less prevalent at more central levels. Cells were also found in N.VIII that responded to tones with a decrease in the spontaneous activity. Kay (1964) and Katsuki et al (1958) observed that there was intermingling of "rate coded" amplitude and frequency parameters. Katsuki (1958) reported that for many cells there existed a rough ogive relationship between frequency and stimulus intensity.

In the cochlear nucleus the response characteristics of the cochlear nerve are repeated but are modified and increased in complexity. Most units show a skewed frequency response as do N.VIII cells and many have a spontaneous discharge. Møller (1969) reported that the spontaneous activity of some cells would remain constant for hours while in other cases it would change for no identifiable reason. Many investigators have identified cells that responded with either an increase in spike rate or a decrease in rate depending upon the tone frequency (Kay, 1974; Greenwood et al, Møller, 1971, 1975).

As Møller (1969a) stated, often units were encountered that were characterized by an excitatory frequency band "sandwiched" between inhibitory bands. A few neurons showed bi-modal frequency response characteristics (Van Gisbergen, Grashuis, Johonnesna, and Vendrik, 1975), responding equally well to two different frequencies. Units were also identified by the temporal characteristics of their responses (Møller, 1969 a,b). Some cells responded to a constant tone with a high sustained rate of firing. Others, described as transient cells, reacted to tone onset with a burst of activity which then fell rapidly to a much lower rate. The amplitude of the initial burst of these cells appeared to be dependent upon the duration of the silent interval between successive stimuli.

The specific relationships between the inhibitory excitatory characteristics, the temporal properties and the spontaneous activity of cochlear nucleus cells gives rise to a variety of complex response types. Based on these parameters, Van Gisbergen et al (1975) have proposed a classification scheme which allows the characterization of functional cell types. A cell is first classified as silent or spontaneously active. It is then subtyped as responding to stimulation with activation, suppression, (spontaneous cells only) or activation and suppression. Each subtype is then further discriminated on the basis of the temporal pattern of response: sustained, transient, build up, or complex (pause). Van Gisbergen's (1975) research showed that sustained cells were

predominant in the VCN while build up and complex units dominated the DCN. Sustained cells were equally distributed. These authors went on to propose that the presence of the more complex response types in the DCN was due to the existence of more potent and widespread inhibitory mechanisms within the DCN. This correlated nicely with the reports of Rasmussen (1966) discussed previously that the DCN has a preponderance of non-afferent synaptic connections. It thus appears that the DCN may be a primary site for interaction between inhibitory and excitatory mechanisms.

Møller (1971, 1972) has demonstrated two phenomena which may be explained by the dynamic interaction of the inhibitory and excitatory influences upon a cell. He found that a tone stimulus that was frequency modulated at a high rate with a center frequency equal to the CF produced a response band width that was narrower than the bandwidth found using tones of single frequencies. One explanation of this effect may be that, for the FM stimulus, the tone frequency must pass through an inhibitory range before reaching the excitatory range of the cell. This could result in narrowing of the bandwidth if the temporal pattern of inhibition and excitation interacted properly. Møller (1971, 1972) also found that sinusoidal amplitude modulation produced maximum modulation of the neural response rate at a modulation frequency of approximately 200 HZ. At this frequency the neural response often was more highly modulated than the stimulus. Presumably this might also be explained by the temporal summation of inhibitory and

excitatory influences.

Such phenomena have prompted the study of the effects of noise upon single unit response characteristics. Møller (1969b) found that responses of transient units to clicks could be inhibited by broad band noise or by pure tones of a frequency slightly above or below the CF. Greenwood et al (1965) demonstrated the masking of the response to a tone at CF by a noise band above and below CF but not including the CF. They noted, consequently, that wide bands of noise which overlap the excitatory and inhibitory response regions are much less effective stimuli for single cells than are narrow bands confined to the excitatory area. Katsuki et al (1958) studied DCN responses to tones with a wide band background noise. They describe the results: when the intensity of noise was increased step by step, the pattern of the spike volley changed little by little and at some moment, instead of such spike volleys, there appeared new irregular spike discharges elicited by the noise.

It might be conjectured that such an effect was due to the increasing potency of inhibition above and below the CF which at some level was able to block the response to the tone at CF, thus producing a new response pattern. This may have been the point at which, for the animal, the noise became more significant than the sound. In any event, this technique produced a response which was dependent on both the inhibitory and excitatory influences impinging upon the cell.

Evidence concerning the functioning of the efferent auditory

mechanisms is sparse. Desmedt (1960) reported that electrical stimulation of the ventral nucleus of the lateral lemniscus decreased the amplitude of evoked potentials in the cochlear nucleus. Pfalz (1973) demonstrated that electrically elicited evoked potentials of the VCN of one side were inhibited by auditory stimulation of the contralateral ear. Thornton and Coleman (1975) investigated the adaptations of cochlear and brainstem auditory evoked potentials in humans. They proposed a neural model of the adaptation process in which some adaptation occurred at all levels of the auditory system. If adaptation is dependent on efferent mechanisms, a not unreasonable proposal, then this model would correspond well with the anatomical evidence for centrifugal inputs at several processing rates.

Based on the information that has been presented above, in particular the studies of Bradley and Kay, the current research was designed to examine the following general hypothesis:

> There exists a modulatory input to the classical auditory pathways at the level of the cochlear nucleus. The action of this input alters the response parameters of the neurons of the cochlear nucleus and is dependent at some stage upon the activity of serotonin.

It is interesting to note that the methodology of studies of this type are often completely devoid of explicit statistical analyses or make use of little more than the most elementary

statistical approaches. For example, in their studies of the response characteristics of the cochlear nucleus, both Møller (1972) and Van Gisbergen et al (1975) present visual displays or graphs of the response characteristics and leave the analysis of these measures up to the reader with the help of a verbal discussion in the text. On occasion authors will use histograms or averaging techniques but most often data is merely presented for visual inspection. One exception is the interesting use of cross-correlational techniques by Møller (1975) to evaluate the input/output relationships of the cochlear nucleus.

In many investigations of the type to be described in this paper, there are no attempts to quantitatively compare the experimental and control samples with a statistical test. Thus a second purpose of this research was to evaluate the applicability of statistical methods to data of the genre generated by this experiment. In particular, an investigation was made of the validity of the assumption of parametric statistics that the sample observations are selected from normally distributed populations of homogeneous variance (Siegel, 1956).

Methods

In the present electrophysiological study, 22 Sprague-Dawley derivative, male, albino rats obtained from West Jersey Biological Farm (Wenonah, N.J.) were employed. Upon delivery they were housed singly in a controlled environment, temperature regulated to approximately 20 $\pm$ 2.0^{o} C. A 12 hour light (8 a.m. to 8 p.m.)/12 hour dark day night cycle was instituted and Purina Rat Chow (15 gms./day) and water (ad lib) were provided. All rats weighed between 230 and 310 grams at the time of surgery.

The following surgical technique was used to gain access to the cochlear nucleus. Rats were initially weighed and injected with atropine sulfate (0.8 mg/kg I.P.) (Eli Lilly & Co.) to help control excess mucous secretions. Ten minutes later an injection of sodium thiopental (50 mg/kg I.P.) (Abbott Laboratories) was administered. Due to the long duration of the surgical procedure (45-60 minutes) and the rapid time course of the anesthetic, an additional dose of sodium thiopental (25 mg/kg I.P.) was administered 25 minutes after the first anesthetic injection. It should be noted that Yoo, Lee, and Yoo (1968) indicated that administration of sodium thiopental caused only slight changes in brain 5-HT levels. Furthermore Bradley and Key (1958) found that any central effects of atropine sulfate involved only diffuse thalamic projections and had no effect on brainstem structures.

Following the first anesthetic injection, the head and abdomen

were shaved. Subcutaneous needle electrodes were inserted on either side of the chest and on either side of the head, just rostrally to the ears. The outputs of these electrodes were displayed on two channels of a Grass Model 7 Polygraph, thus allowing constant monitoring of heart rate and E.E.G. An anal temperature probe (Yellow Springs Instrument Co.) allowed monitoring of the body temperature.

Prior to the beginning of surgery a special syringe was prepared for the purpose of injecting the experimental drug. An 18 gauge syringe needle was cut in half. The pieces were then inserted into opposite ends of a length of tightly fitting polyethylene tubing, thus providing a flexible extension between the syringe body and the needle tip. This latter portion was inserted in the peritoneum of the rat.

The rat was mounted in a stereotaxic holder. A midline incision was made extending from between the ears caudally for approximately 2 cm. The wound margins were retracted laterally with hemostats and the underlying periosteum was scraped away from the skull. The open wound was then irrigated with a local anesthetic, procaine hydrochloride (Novocain), for five minutes, at which time the solution was drawn off with an aspirator and the bone rinsed once with Ringer's solution. From this point on, care was taken to insure that tissue inside the skull was not contaminated with the anesthetic in the tissues outside the skull.

A dentist's drill was employed to remove a section of cranial

bone above the cerebellum. This section was bounded rostrally by the lambda bone suture and on the remaining sides by the edge of the cranium at the junction of the dorsal bone surface with the lateral and caudal bone surfaces. Bone rongeurs were used to further enlarge the opening as needed.

Throughout the bone removal process it was found wise to have a small peice of Gelfoam available in the event of excess bleeding. It was noted that the transverse venous sinus extends laterally at a point directly under the lambda suture. In the event that this sinus was penetrated during the bone removal, the application of the Gelfoam rapidly prevented large blood loss.

The remaining steps in the surgical procedure were performed with the aid of a Bausch and Lomb stereomicroscope. An incision was made in the dura and pia mater along the lateral and caudal edges of the cranial opening. This provided a meninge flap which was folded upward and anteriorly so as to lie upon the dorsal surface of the skull just anterior to the opening. An aspirator was used to perform this manipulation and to perform a similar manipulation on any blood vessels lying upon the surface of the cerebellum. If retracted forward and held in this position for a few moments the tissue would adhere to the bone surface and remain so attached. By this method, the blood vessels were sealed off, as were any small punctures in the transverse venous sinus at the anterior edge of the cranial opening.

The aspirator was then used to remove the right posterior

lobe and flocculus of the cerebellum and underlying meninges. This allowed visualization of the acoustic tubercle lying just posterior to the cerebellar peduncles. Ventral to the acoustic tubercle lies the dorsal cochlear nucleus, the target site.

Glass micropipettes were constructed using a commercial pipette puller (Industrial Science Associates, Inc.) They were then filled with 3 Molar KCl solution by gently boiling the immersed electrodes for one hour and then allowing tham to cool overnight. A small silver wire was used to make electrical contact with the fluid inside the micropipette and a small peice of clay sealed the top end of the completed electrode. The electrode impedance was determined as follows. A variable resistor was inserted in series with the electrode. The tip of the electrode was immersed in Ringer's solution which, via a silver wire, was connected to the ground lead of a square wave stimulator. The square wave signal was then applied across the series combination of the resistor and electrode. The wave form appearing across the electrode alone was monitored with a high impedance preamp and oscilloscope. The amplitude of the square wave was noted with the variable resistor set to 0.0 ohms. The resistor valve was then increased until the amplitude of the signal appearing across the electrode was one half the original value. At this point the value of the variable resistance was equal to that of the electrode and was thus noted. The impedance of all electrodes

used in the present study ranged between 7 megohms and 20 megohms. Corresponding tip diameters were estimated by microscope to be less than 2 microns.

An indifferent electrode was constructed by manually sharpening one end of a short length of silver wire. The opposite end was soldered to a length of small diameter brass tubing to provide a holder. This electrode was inserted into the brainstem at the floor of the fourth ventricle medial to the acoustic tubercle. The micropipette electrode was inserted into the cochlear nucleus with the aid of a hydraulic microdrive unit (David Kopf Instruments, Model 1207B).

Auditory stimuli were generated by combining the outputs of a Grason-Stadler Model 901B white noise generator and a General Radio Co. Type 1304-B beat-frequency audio generator. The output of the latter was coupled through a Grason-Stadler electronic switch and a Grason-Stadler interval timer, thus providing a composite signal consisting of a continuous white noise component plus bursts of single frequency tones. This signal was coupled to a Realistic MC 1000 high fidelity speaker which was mounted over an opening in the recording chamber. The recording chamber itself was constructed of two layers of 0.95 cm. plaster board separated by a 1.9cm layer of fiberglass insulation material. The outside deminsions were 91.5 cm in width, 92.0 cm in height, and 91.5 cm in depth. A screened enclosure measuring

76.0 cm on a side was placed inside this chamber in order to provide electrical shielding from possible sources of interference. The space between the screen enclosure and the chamber walls was filled with crumpled paper so as to minimize standing waves within the chamber when the hinged front doors were closed.

Frequency calibrations were performed with a General Radio Co. Type 760A sound analyser. The direct reading dial of the beat-frequency audio generator was found to be accurate to within ±7.5%. Sound pressure level measurements were performed with a General Radio Co. type 1551C sound-level meter. A cable extension was added to the microphone of this instrument in order to allow continuous monitoring throughout the experiment of the sound pressure levels within the recording chamber. From a preliminary study it was found that the following stimulus intensities provided the most satisfactory response range:

	ambient chamber noise	=	34 dB
white noise +	ambient chamber noise	=	46 dB
tone + white noise +	ambient chamber noise	=	54 dB

All sound pressure levels are given in dB re. 0.0002 ub.

Potentials from the microelectrode were amplified by a Grass Model P16 microelectrode amplifier. The output of this amplifier was A.C. coupled to a buffer amplifier with adjustable gain (Med Associates Inc. ANL 100) and fed to a bandwidth limiting filter (Krohn-hite 330N). The lower 3dB cutoff frequency was normally 200HZ while the upper 3dB cutoff frequency was 6KHZ.

The filter output was coupled to one trace of a dual channel oscilloscope (Tektronix 502A) and was converted to an audio signal with a Grass audio monitor (AM 4). The filter output was also routed to a custom built amplitude "window" discriminator. This unit, the construction of which is described elsewhere (DeYoe & Foster, 1976), was used to determine the number of action potentials (spikes) produced by a particular neuron during each second. When necessary (rarely in this application) the discriminator "window" could be adjusted so as to count spikes of only one average amplitude. Such spikes are likely to originate from only one cell. The discriminator provided three separate output modes. A direct reading decimal display allowed on line monitoring of the actual spike count. A ramp wave form output was also provided. The amplitude of the ramp was proportional to the spike count and was reset to zero at the end of every one-second sample. The resulting signal as displayed on one channel of a polygraph recorder (Grass Model 7) results in a sawtooth waveform of varying amplitude and one-second period. On another channel of the polygraph time marks and "stimulus on" marks were recorded. The third output mode of the discriminator provided the spike count in binary coded decimal. This information was sent to an Altair 8800 microcomputer which stored the data until the termination of the day's recording. The data could then be printed out as decimal

numbers and also stored on magnetic tape.

Synchronization of stimulus tone onset with the beginning of a discriminator sample period was provided by deriving a synchronization pulse from the electronic timer (Psionix 1248A) which controlled the discriminator sampling cycle.

The discriminator also provided an output pulse whenever a spike was counted. This signal was fed to the second channel of the oscilloscope displaying the bandpass filter output. By this method each counted spike could be identified. Polaroid photographs of the oscilloscope display were obtained with the Tektronix C-27 camera attachment. A camera attachment to the Bausch and Lomb microscope provided photographs of the location of electrode insertion. The zoom adjustment of the microscope was set to provide an overall magnification of 125x.

All rats were pretreated with DL-p-chlorophenylalanine (Sigma Chemical Co.) suspended in 0.5% agar solution. An injection of 300 mg/kg I.P. was given to each rat approximately 48 hours before the recording procedure so as to provide an initially low level of brain 5-HT. 5-hydroxy-L-tryptophan (5-HTP) (Sigma Chemical Co.) was suspended in a 0.5% agar vehicle. Two drops of Tween 80 (Polyoxyethylene Sorbitan Monooleate; Sigma Chemical Co.) were added to each 20 ml volume of solution to enhance dispersion of the drug. Control solution con-

sisting only of 0.5% agar, Tween 80 and distilled water was also prepared. The 5-HTP solution or control solution was administered at the appropriate time from outside the recording chamber with the aid of the modified syringe described earlier. Filling of this syringe with control solution was straightforward. An added precaution was taken in the case of the 5-HTP suspension. After filling the syringe and tube with the 5-HTP suspension, 0.05 cc of control solution provided a "buffer" zone which prevented the premature entry of 5-HTP into the rats' circulation. The 5-HTP was given at a dosage of 50 mg/kg and an equal volume plus 0.05 cc of the control solution was administered under the control condition.

The typical experimental recording procedure was as follows. The microelectrode was advanced into the cochlear nucleus until a cell was encountered which responded to either experimenter generated sounds (whistles, squeaks, kissing sounds) or instrument generated stimuli. The frequency of the tone portion of the experimental stimulus was varied until a frequency, the characteristic frequency, was found which produced a maximum increase in the firing rate. This frequency was determined by experimenter judgment based on the auditory monitor signal and the polygraph pike count record. Rarely was such a judgment difficult or ambiguous. The sound pressure levels at this frequency were checked to insure uniform amplitude regardless of frequency. Stimulus presentations which were under manual control during the above proce-

dure were switched to automatic control. This provided 1.0 second bursts (5 msec. risetime) of tone plus white noise separated by 5 sec. of white noise alone. Samples of the spike rate were taken every second as described above.

Recording proceeded in this manner for at least ten minutes. 5-HTP or control solution was then injected and recording continued for another hour or until the cell was lost.

In several rats this procedure was supplemented by recording from three or four different cells before the drug injections and three or four more after the above procedure. These cells were recorded for short periods (approximately two minutes) providing approximately 20 tone presentations for each cell.

A photographic record of the oscilloscope waveforms was obtained for most cells. The characteristic frequency was recorded and an estimate of the penetration depth for each cell was made. A photograph was made of the electrode location at the end of the day's recordings.

Results

22 rats weighing between 240 and 310 grams were subjected to the experimental procedures. All recordings were made between February 13 and March 20, 1976. In all cases the actual records were made between the hours of 11:00 a.m. and 4:00 p.m. Of the 22 rats that underwent the experimental procedures, 9 yielded data of sufficient quality to be included in this study. 69 cells in all were encountered of which 18 have been chosen for discussion. Of these latter 18 neurons, 10 were obtained from one rat. Of the remaining 8 rats used in this study, each one yielded one suitable cell. Those cells not included in this discussion were rejected because they did not yield recordings of sufficient duration or consistency. Those cells from which data were obtained before and after the drug manipulations produced recordings ranging in duration from approximately 20 minutes to one hour. Criteria for the identification of a single cell response versus a multiple cell response were experimenter judgments of the consistency of spike amplitude and form based on the oscilloscope display of the response waveforms.

Figure 4 illustrates the spatial distribution of electrode penetrations into the cochlear nucleus as well as the gross anatomical location of the acoustic tubercle (anatomy after Minckler, 1972). This distribution is a summary of the information

Figure 4

Physical Location of the Acoustic Tubercle (incl. Cochlear Nuclei) Showing Locations of Electrode Penetrations (Dorsal View with Cerebellum Removed)

(· = precise location, x = approximate location)

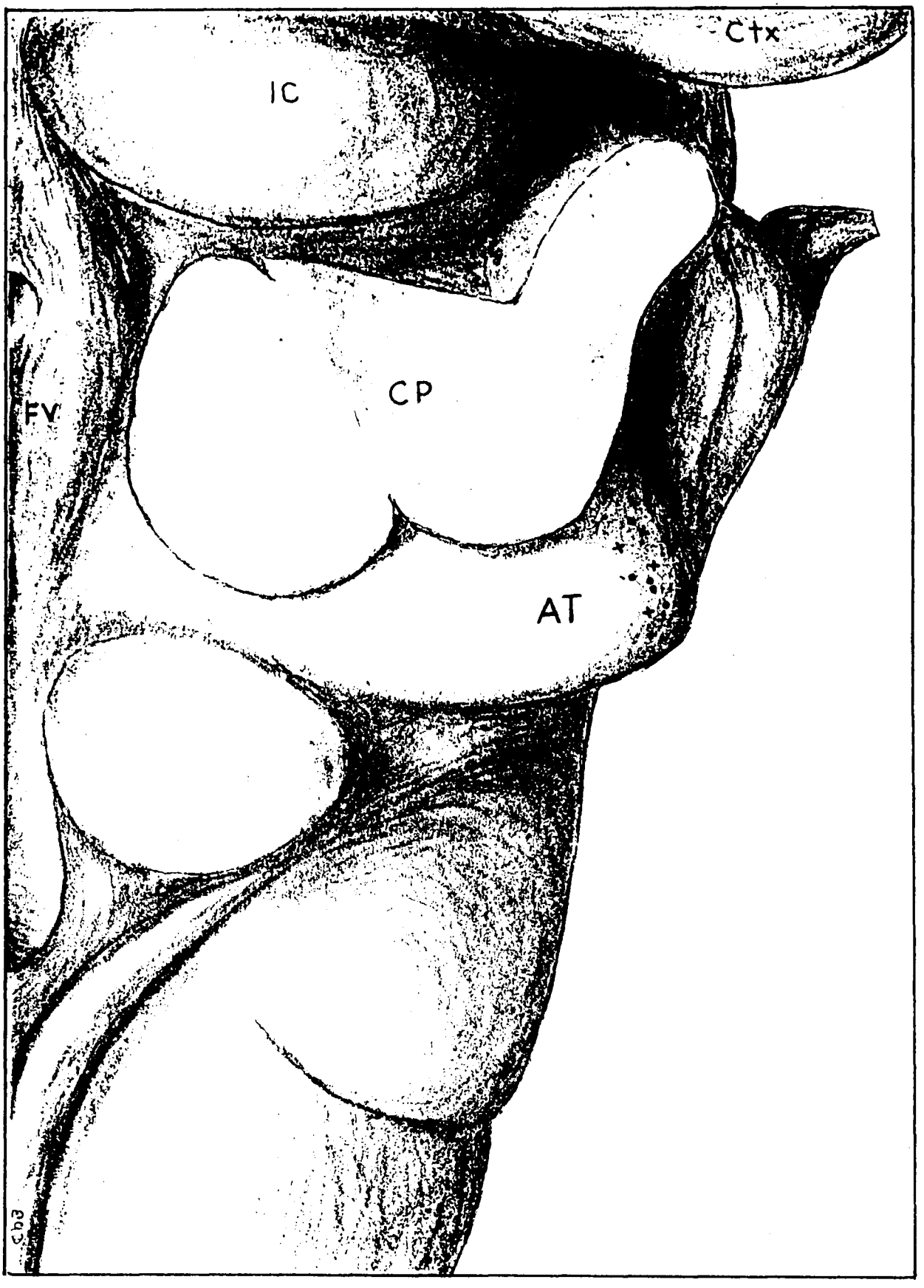
AT - Acoustic Tubercle

CP - Cerebellar Peduncles

CTX - Cerebral Cortex

FV - Floor of 4th Ventricle

IC - Inferior Colliculus


Ctx
IC
CP
FV
AT
Cb3

taken from the microscope photographs of each recording site. Due to the poor quality of some of these photographs, the locations of several penetrations are only approximate and are marked by crosses. The maximum depth of each penetration from point of contact with brain tissue ranged between 0.30 mm to 1.63 mm. The angle of penetration was vertical in the coronal plane. In the sagittal plane the angle of insertion was 20.0^{o} from the vertical, thus causing the electrode to pass in a slightly rostral direction.

The characteristic frequencies (CF) of the 18 cells chosen for analysis ranged between 700 HZ and 14.2 KHZ. The median CF was 3.5 KHZ for all cells but was 7.2 KHZ for the 9 units that were recorded both before and after the drug manipulations. Cells of different CF's were encountered in an ordered sequence within each electrode penetration. Cells exhibiting high CF's were usually found shortly after initial penetration. Cells of decreasing CF were then located as the electrode was advanced. The spike rate versus stimulus frequency characteristics of the cells were varied. Some cells showed only a simple excitatory range. Others showed both an excitatory and inhibitory range while in one case an excitatory region was discovered sandwiched between inhibitory regions. In this latter case the cell responded maximally at 4.60 KHZ and minimally at both 2.4 KHZ and 5.15 KHZ. Most cells showed a much more rapid roll off on the high

frequency side of the CF than on the low frequency side.

Spike rates for different cells varied considerably. The term spontaneous rates, as used in this paper, will refer to unit responses in the presence of a 46 dB ambient plus white noise background as described earlier. These responses will also be referred to as the noise response. The response to tone plus white noise plus ambient noise will be known as the signal response. During the recording process, units responding only by inhibition of their spontaneous rate were encountered but were not studied. The vast majority of units were characterized by a distinct noise response, although a few cells were found that seemed to show no such response for periods of several seconds. Spike rates for different cells varied considerably. Spontaneous rates as high as 119 spikes per second (sps.) were recorded, although values above 80 sps. were rare. Signal response rates as high as 320 sps. were found but the majority of cases ranged between 10 and 100 sps.

Representative Polaroid photographs of the filtered spike wave forms as displayed on the oscilloscope are shown in Figures 5-9. In all photographs the top trace shows the noise response and the bottom trace shows the signal response. Amplitudes are uncalibrated. Sweep speeds are as indicated. Figures 5 and 6 show a control cell before and after the control injection respectively. Such photographs were used to judge the stability

Figures 5 and 6

Response Waveforms of a Control Cell

Fig. 5 - Before Control Injection

Fig. 6 - After Control Injection

(Vertical - uncalibrated)

(Horizontal - 50 msec/cm.)

(Dots above each trace indicate those spikes that were counted.)

Figure 5

Figure 6

Figures 7 and 8

Fig. 7 - Response Waveform of an Experimental Cell (Horizontal - 50 msec./cm.)

Fig. 8 - Same Response as in Fig. 7 but at a Higher Sweep Speed (Horizontal - 2 msec./cm.)

(Vertical - uncalibrated)

(Dots above each trace indicate those spikes that were counted.)

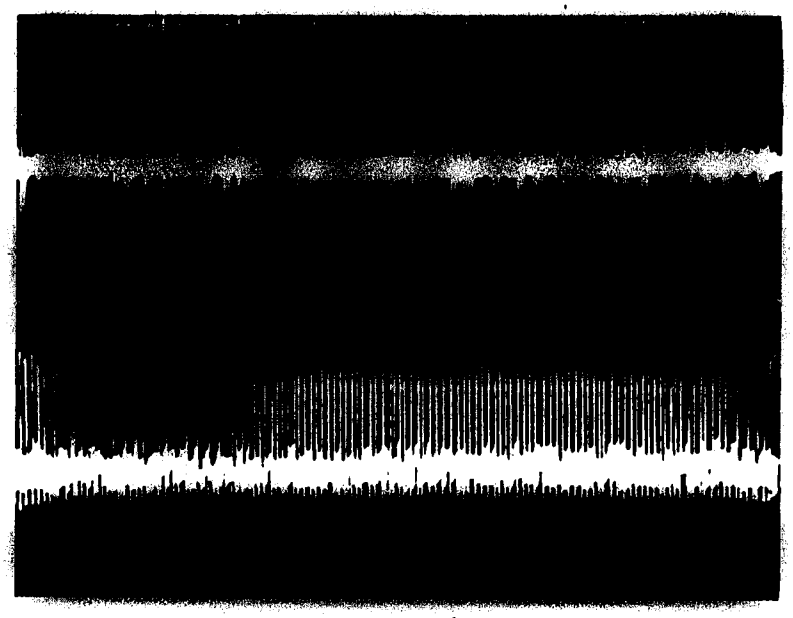


Figure 7

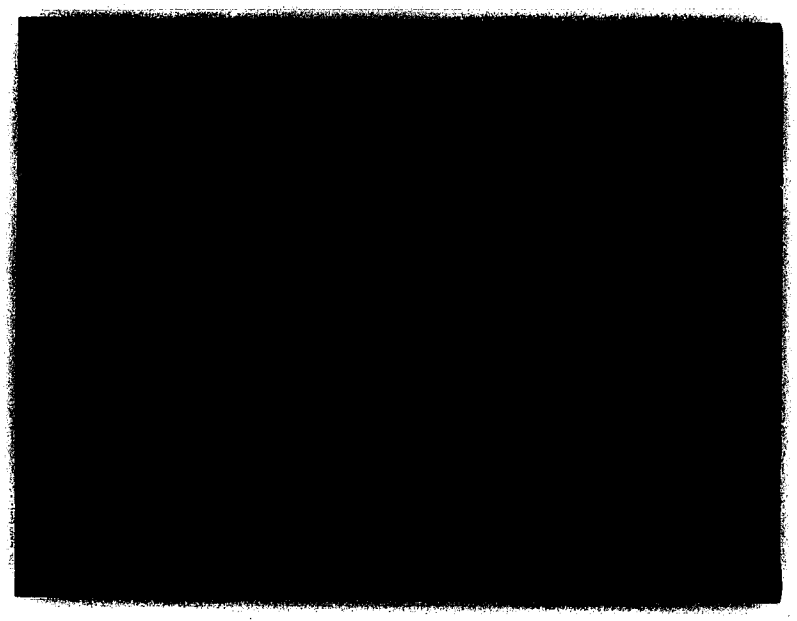


Figure 8

Figure 9

Response Waveform of a Control
Cell Showing Burst at Tone Onset

(Vertical - uncalibrated)
(Horizontal - 50 msec./cm.)

(Dots above each trace indicate those
spikes that were counted.)

Figure 9

of the cell penetration and to verify that only a single cell was being studied. They were not used to make comparisons between control and experimental groups. Figure 7 illustrates an experimental cell. Figure 8 presents the same cell response at a higher sweep speed, thus allowing visual inspection of the details of the filtered spike wave form. Uniformity of the spike shape verifies that only one cell is being recorded. Figure 9 shows a control cell with an initial spike burst at tone onset depicted in the lower trace. This initial burst followed by a lower sustained rate was typical of many cells studied.

A third response measure was calculated from the ratio of the signal response to the noise response and will be called the signal to noise response ratio (SNRR). This response parameter provides a measure of the contrast coding capability of the cell. SNRR values between 1.65 and 1336.0 were found, though in all but three cases this value was below 85.0.

Figures 10-12 present graphs of the three spike response measures (noise response, signal response, SNRR) versus blocks of 60 samples. As described earlier, one sample consists of five seconds of noise plus one second of tone. Thus each block represents the mean response over a six-minute period. Note that the vertical axis is graduated in terms of amplitude relative to the initial point of each graph and thus has not been assigned absolute values. In addition, the initial points of each graph

have been arranged to be one vertical division apart. The graphs were drawn in this manner to allow visual comparisons of the response values obtained before and after the experimental injection. Comparisons should only be made of the deviations relative to the initial point. Absolute values should not be contrasted. Absolute values are given in Appendices A and B. (Note: two graphs of the Mean SNRR have been scaled.) The experimental injection was administered at point 0 on the horizontal axis. A negative block value indicates samples taken before the drug injection. Positive block values correspond to samples obtained after injection. Nine cells were studied in this manner: 5 experimentals and 4 controls. Examination of these graphs reveals no consistent differences among the mean response measures of control and experimental cells.

Figures 13-15 present graphs of the standard deviations of each response measure per 60 sample block. These plots allow study of changes in response variability as a result of the drug injections. Note that for one cell, the noise response was so low that the standard deviation of the SNRR could not be calculated and has thus been omitted. Examination of these graphs also fails to reveal any consistent differences among the standard deviations of the control and experimental cells.

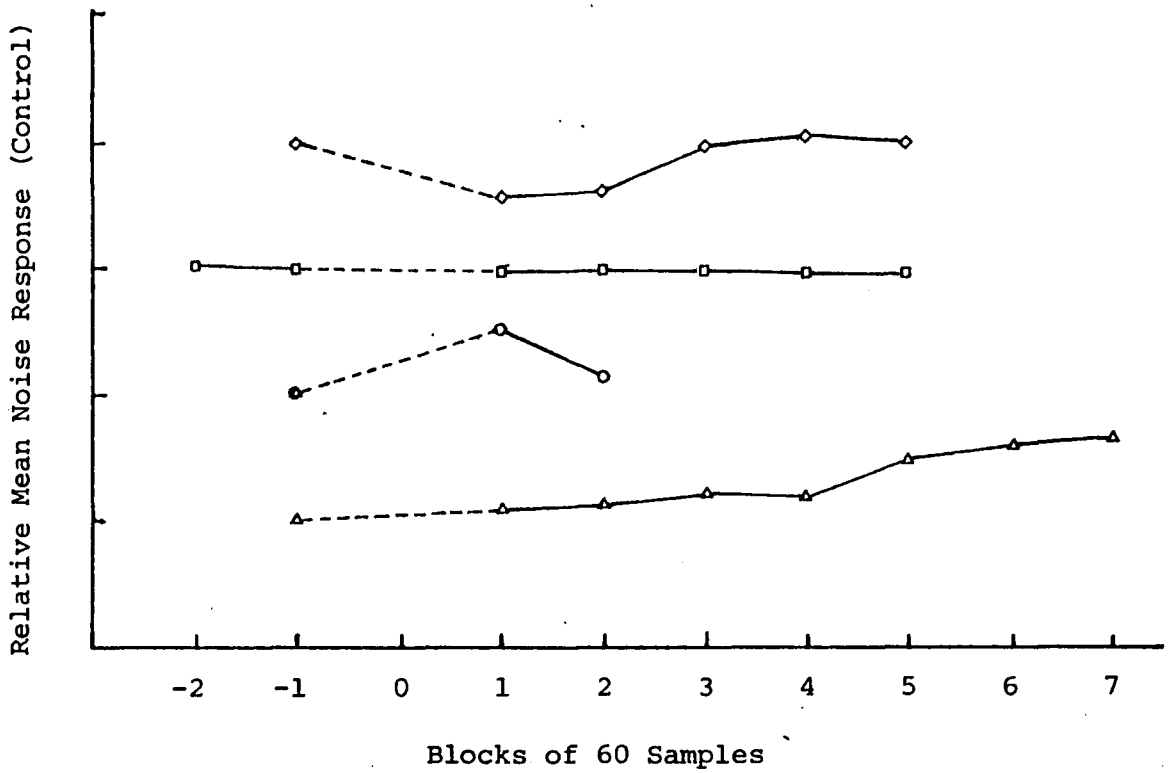
To complement the information presented in the graphs, difference scores were calculated for each cell. This was accom-

Figure 10

Graph of Mean Noise Response
of 4 Control and 5 Experimental Cells

vs.

Blocks of 60 Samples


Relative Mean Noise Response (Control)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

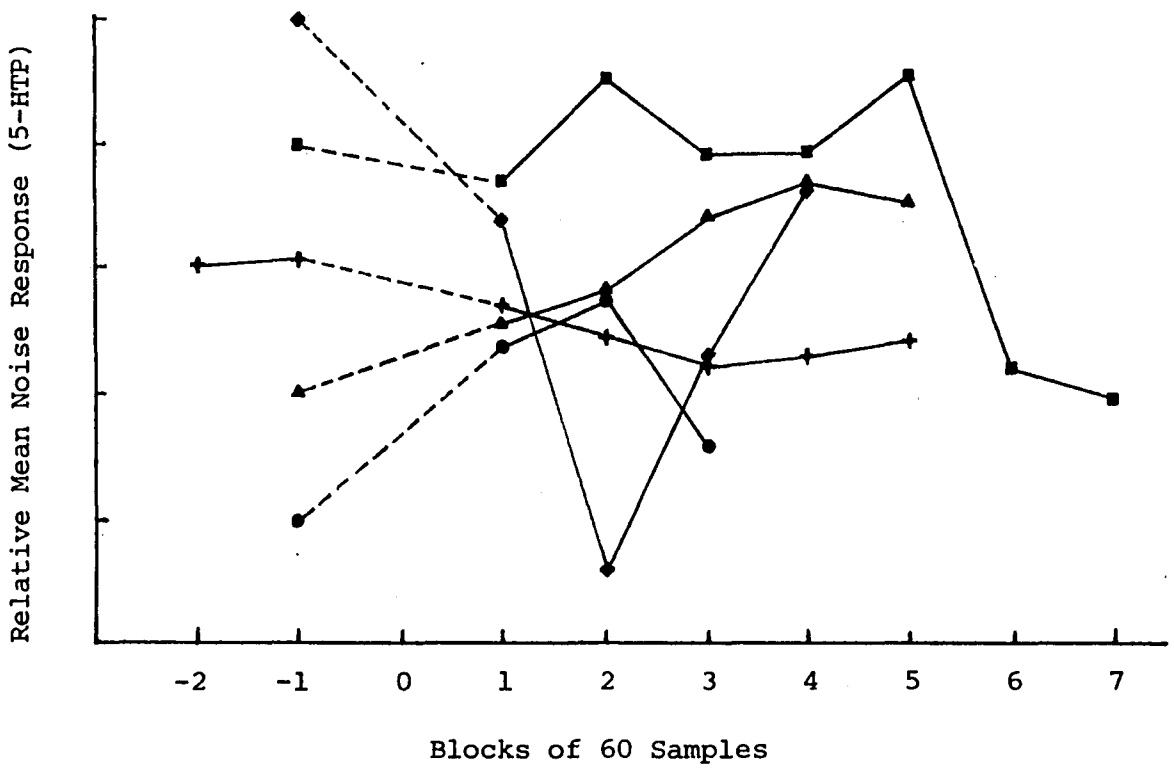
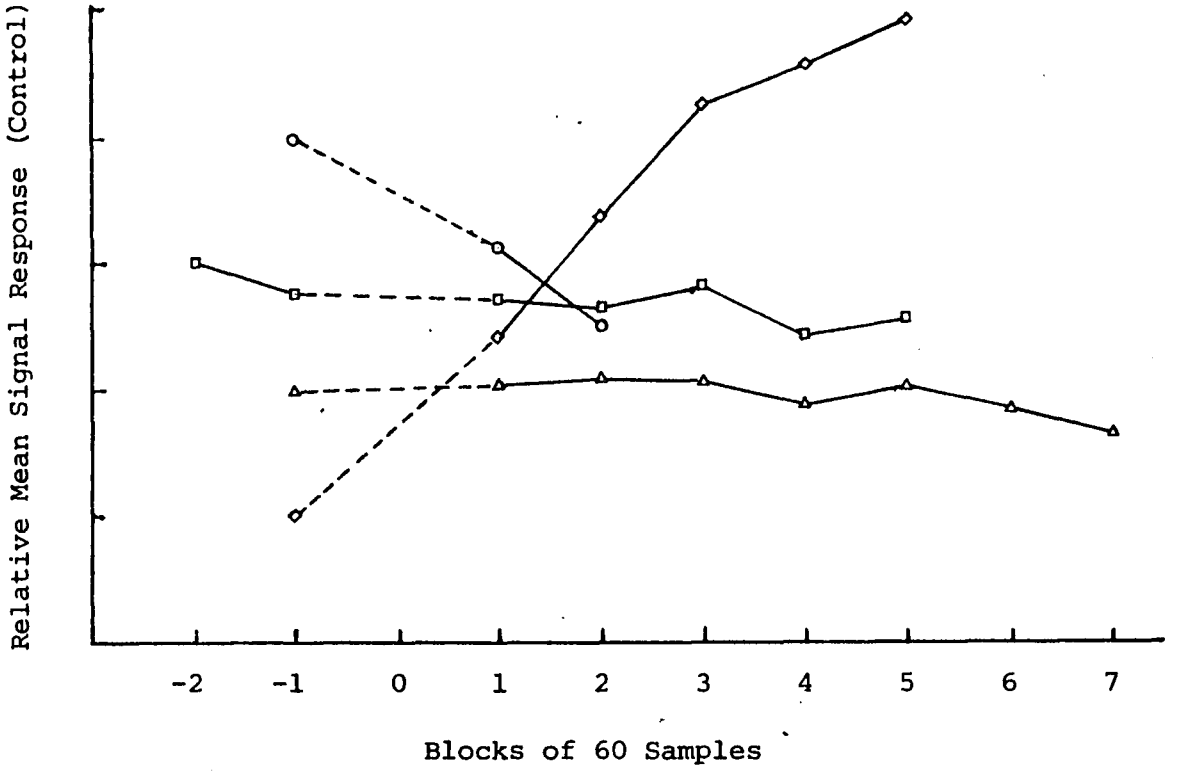

Relative Mean Noise Response (5-HTP)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

Figure 11

Graph of Mean Signal Response
of 4 Control and 5 Experimental Cells

vs.

Blocks of 60 Samples


Relative Mean Signal Response (Control)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

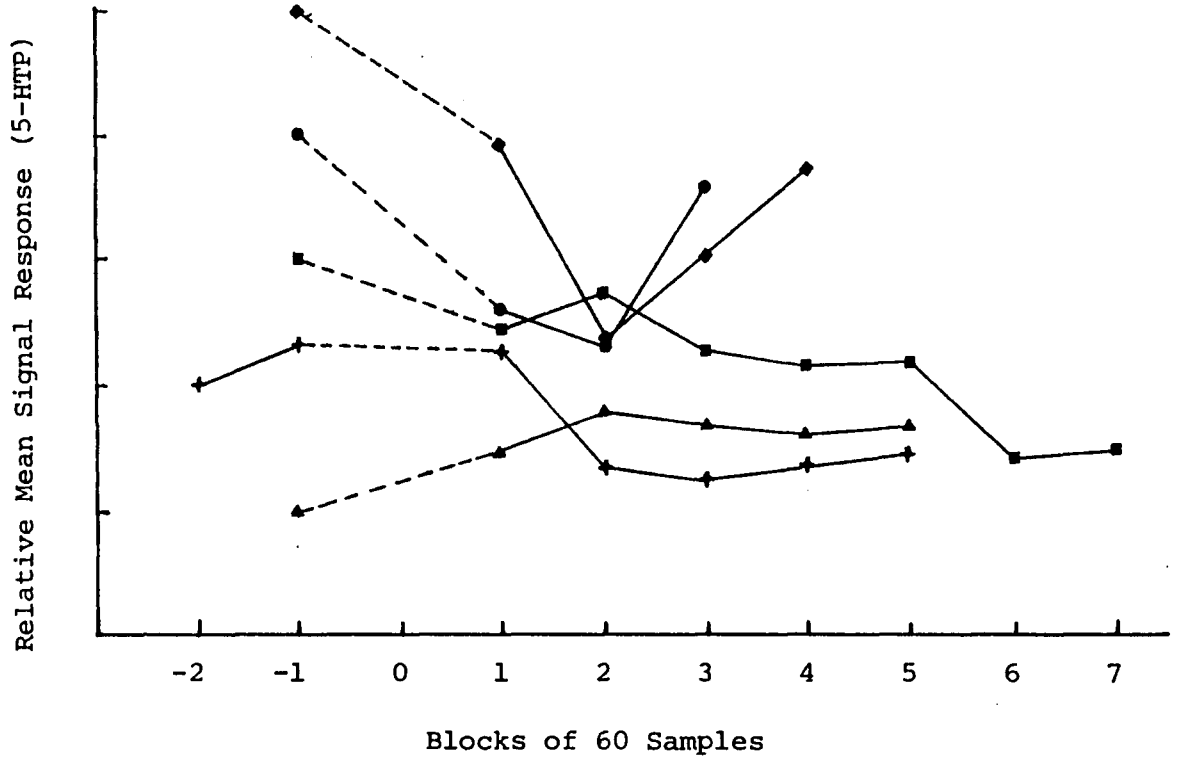

Relative Mean Signal Response (5-HTP)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

Figure 12

Graph of Mean SNRR
of 4 Control and 5 Experimental Cells

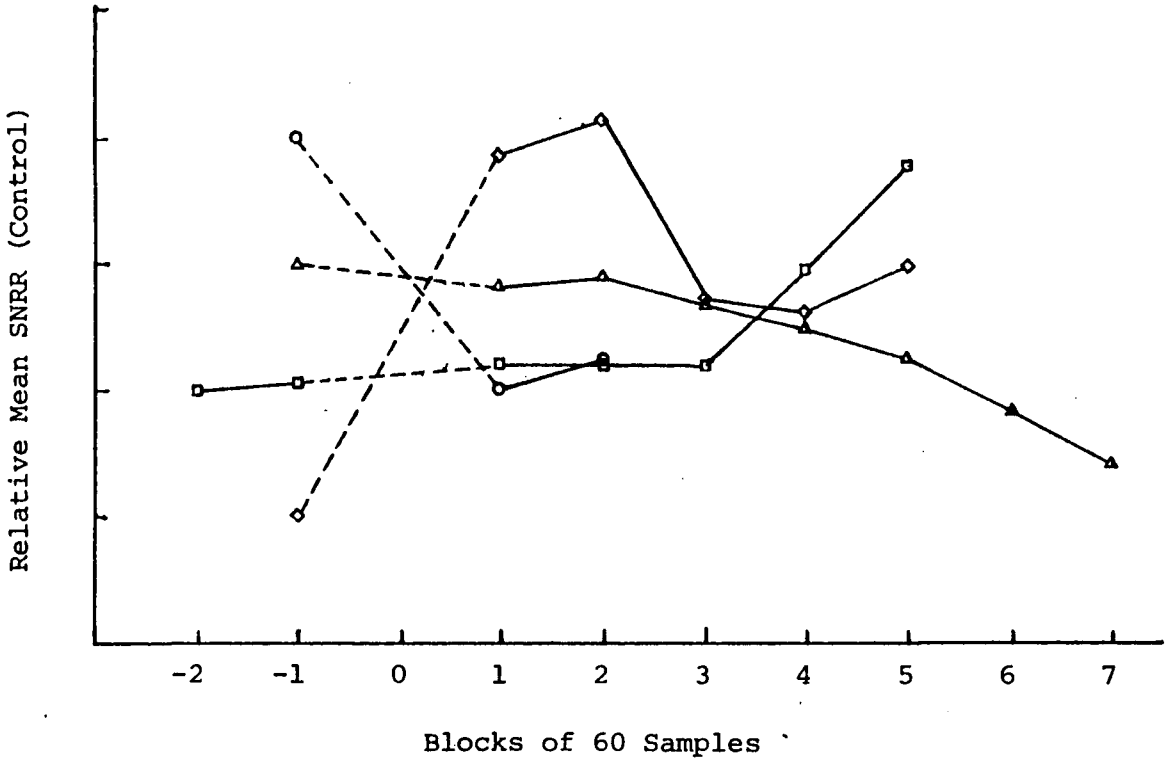
vs.

Blocks of 60 Samples

The following graphs have been scaled to allow representation on a single axis:

—□— - scale factor = 10^{-3}

—◇— - scale factor = 10^{-1}


Relative Mean SNRR (Control)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

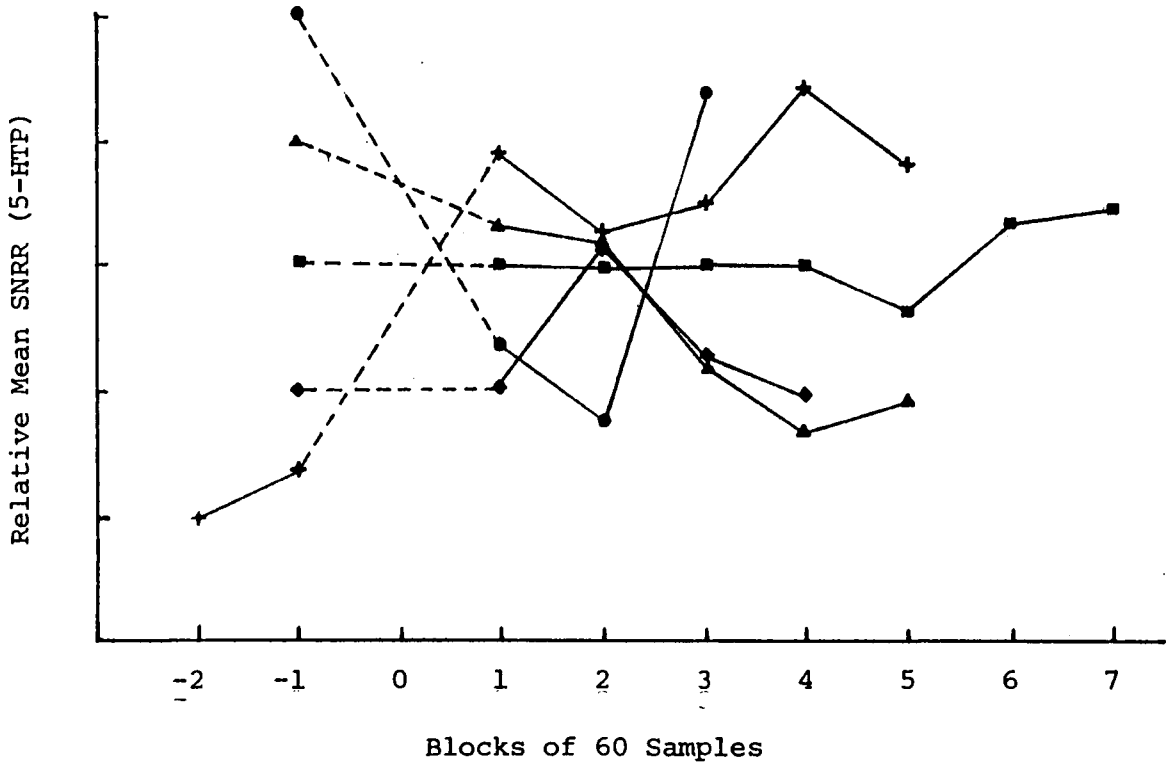
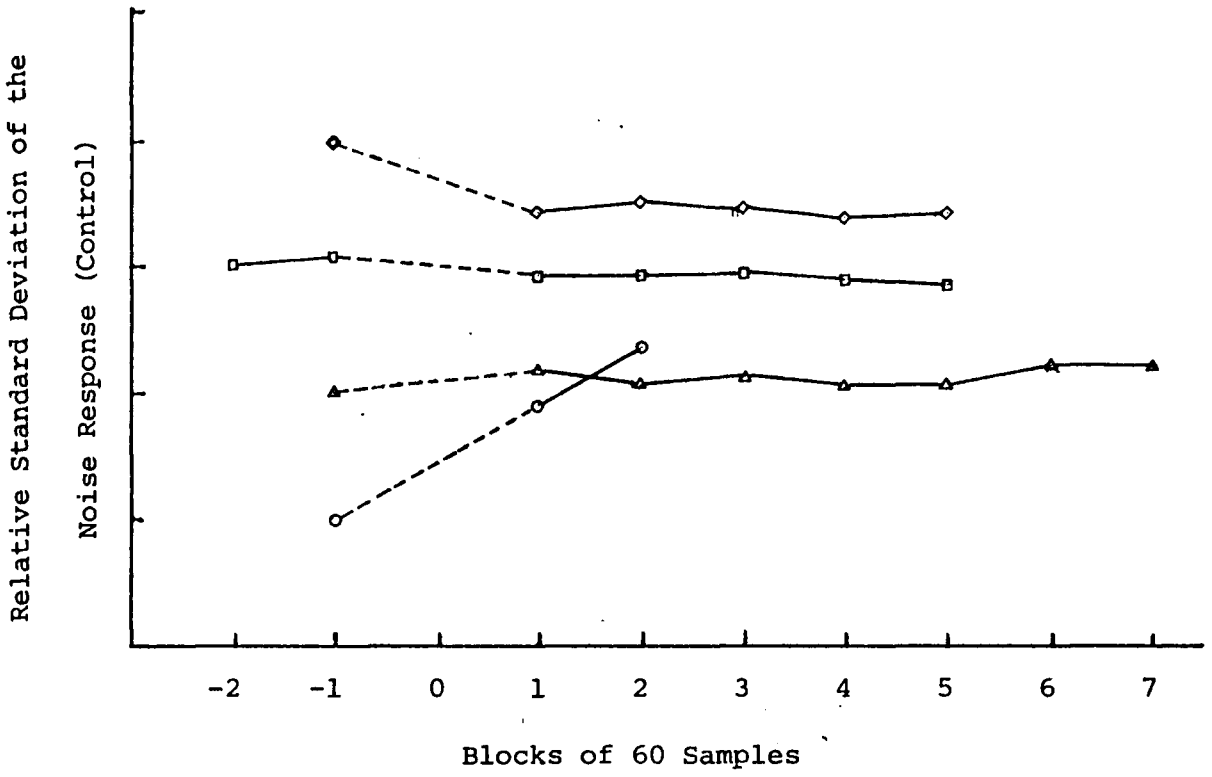

Relative Mean SNRR (5-HTP)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

Figure 13

Graph of Standard Deviation of Noise Response
of 4 Control and 5 Experimental Cells

vs.

Blocks of 60 Samples


Relative Standard Deviation of the
Noise Response (Control)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

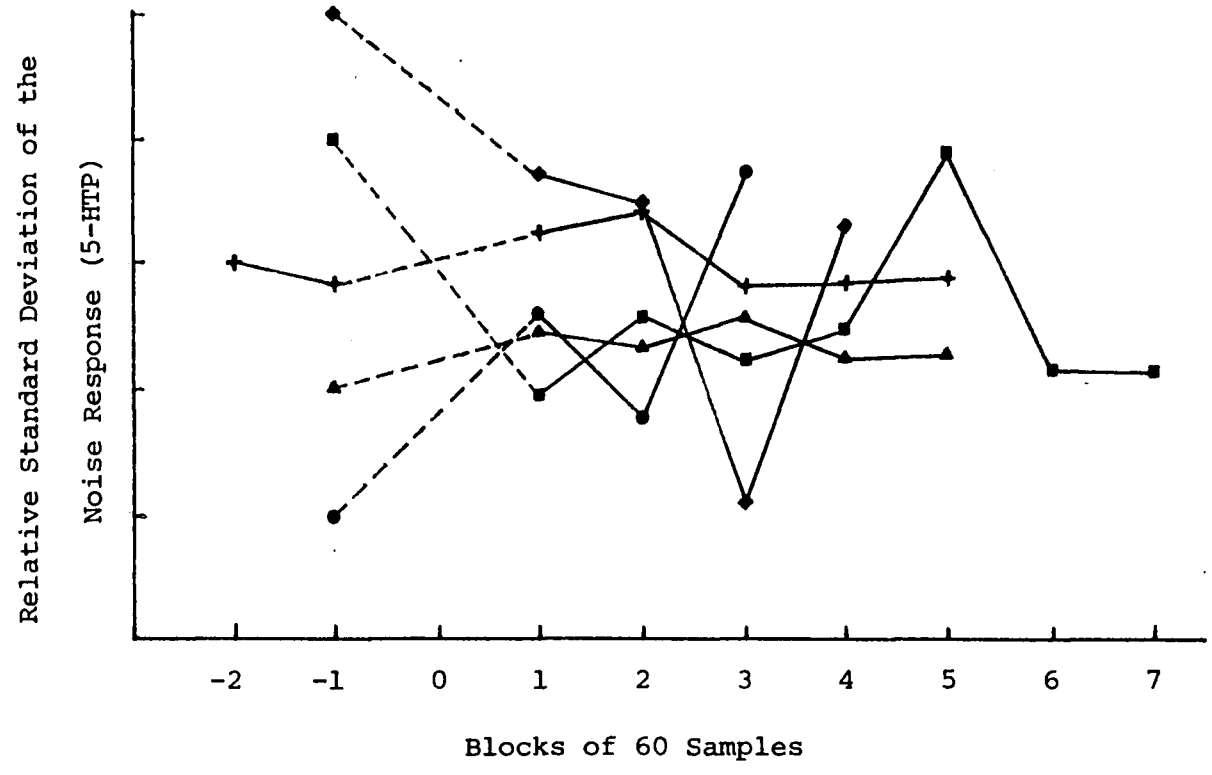
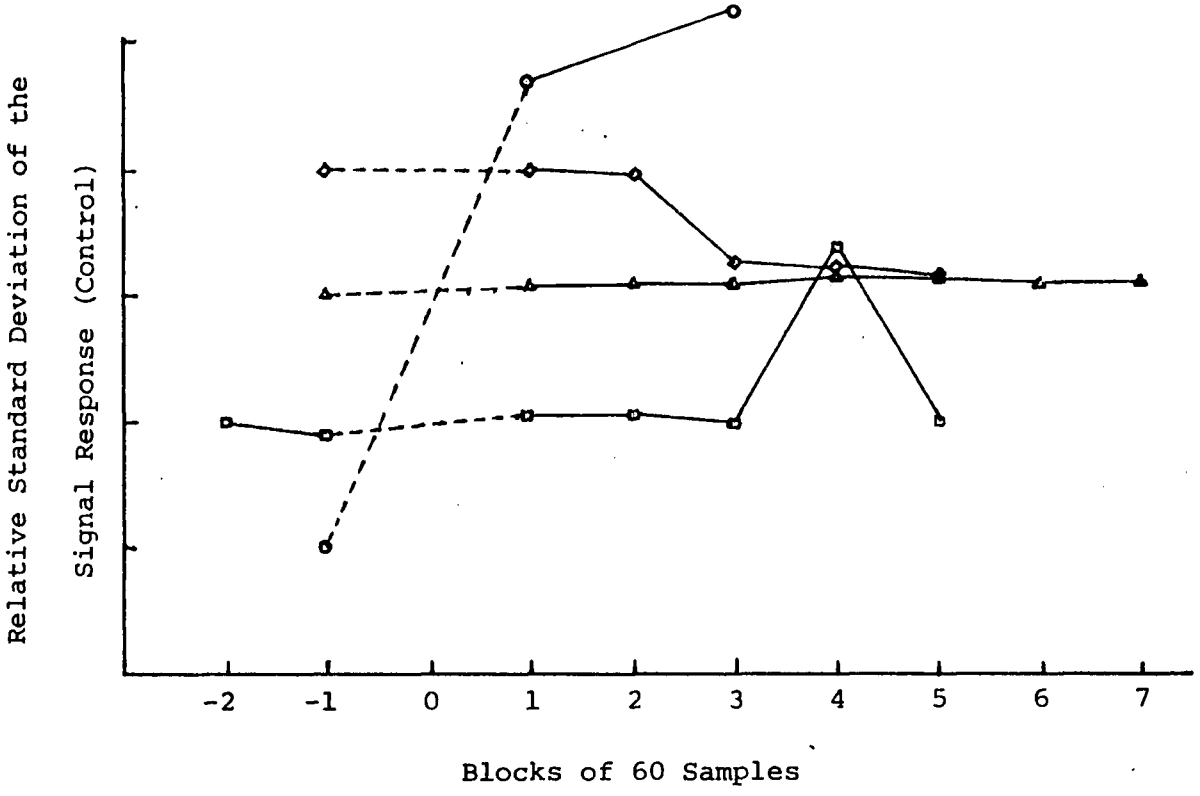

Relative Standard Deviation of the
Noise Response (5-HTP)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

Figure 14

Graph of Standard Deviation of Signal Response
of 4 Control and 5 Experimental Cells

vs.

Blocks of 60 Samples


Relative Standard Deviation of the
Signal Response (Control)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

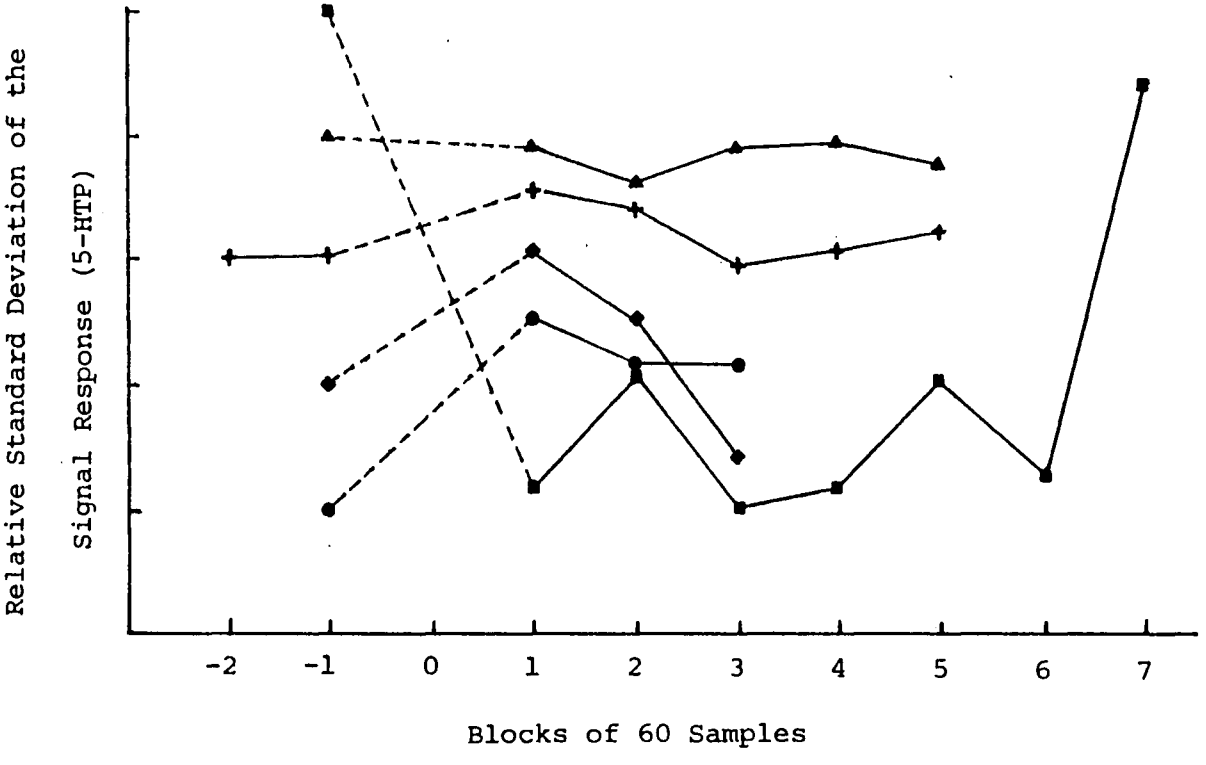
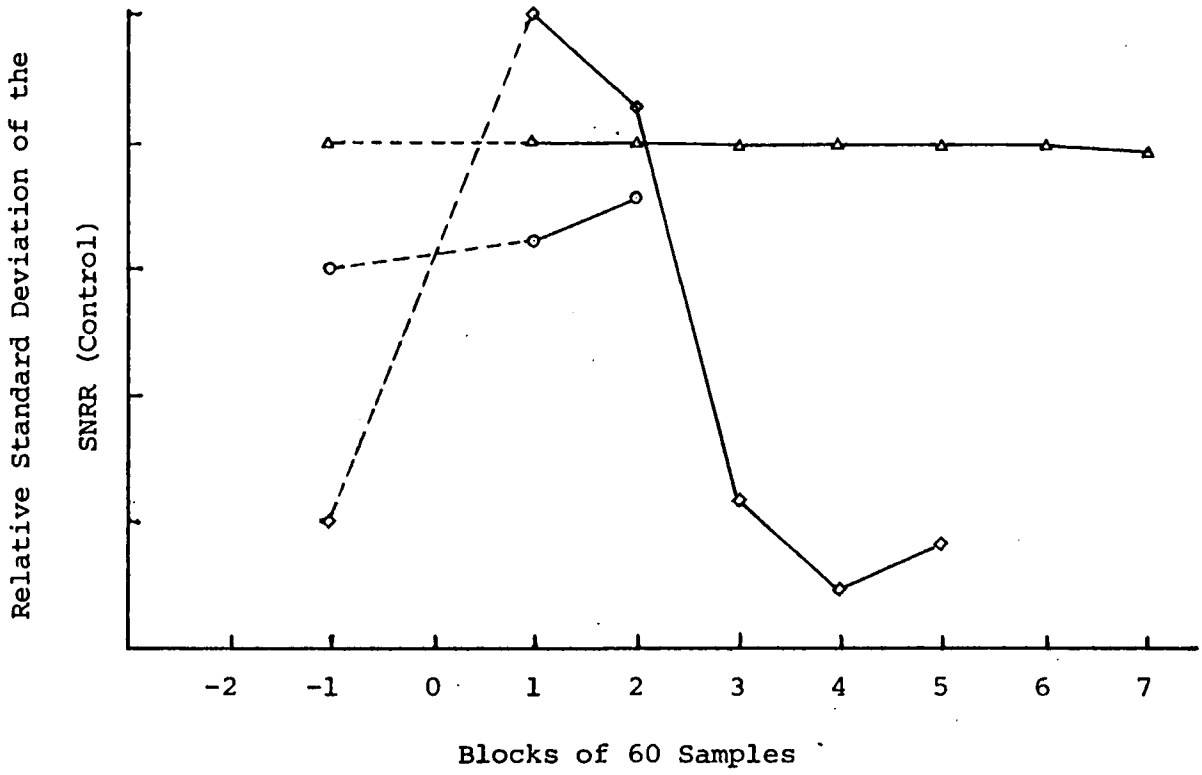

Relative Standard Deviation of the
Signal Response (5-HTP)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

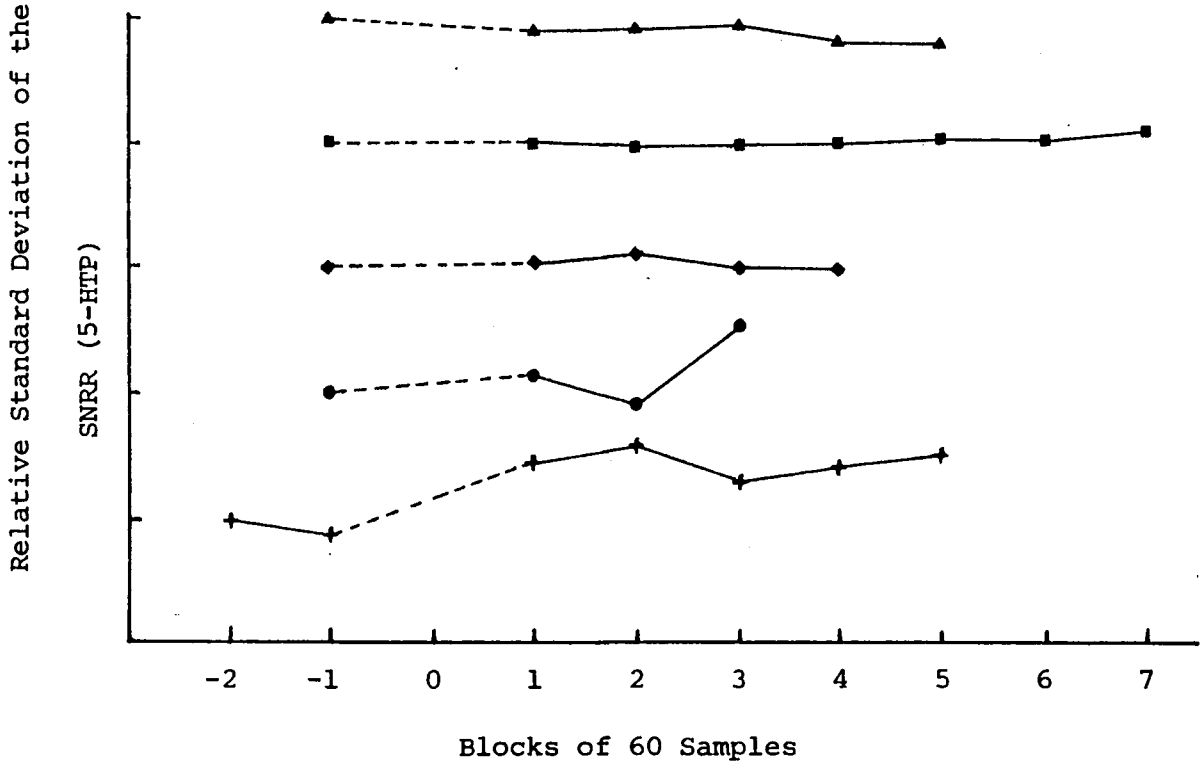
Figure 15

Graph of Standard Deviation of the SNRR
of 3 Control and 5 Experimental Cells

vs.

Blocks of 60 Samples


Relative Standard Deviation of the
SNRR (Control)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples


Relative Standard Deviation of the
SNRR (5-HTP)
-2
-1
0
1
2
3
4
5
6
7
Blocks of 60 Samples

plished by computing the mean and standard deviation of each response measure for the period before injection and for the period after injection. The latter score was then subtracted from the former. These scores are reported in Appendix A. Finally a Mann-Whitney U analysis was employed to test for differences between the experimental and control cells on the basis of these scores. No significant differences were found ($p > .05$; $n_1=4$, $n_2=5$).

Since it was not known whether the response samples obtained were normally distributed, it was felt that the response samples might differ in some ways that were not accounted for by the means or standard deviations of the response measures. Consequently, the skew and kurtosis of each response measure were calculated for the periods before and after drug injection. Skew is defined as the third moment about the mean of the sample distribution whereas the kurtosis is defined as the fourth moment about the mean. Again difference scores were obtained and the Mann-Whitney U test applied. No significant differences were found between control and experimental groups ($p>.05$; $n_1=4$, $n_2=5$).

In addition to the 9 cells which were studied before and after the drug injection, 9 other cells were recorded from one control rat. For each of these cells recording persisted for only two or three minutes producing a minimum of 20 samples for each

Table 1

Table 1

Mean Response Measures and Standard Deviations of Single Control Cells Before and After Experimental Injection

Group	Cell No.	Mean (Spikes/sec.)	Standard Deviation
		Noise Response	
Before Injection	1	2.618	.870
	2	.162	.206
	3	.536	.357
	4	.610	.366
	5	.018	.059
After Injection	6	11.927	6.361
	7	4.964	1.063
	8	24.876	1.928
	9	27.038	39.073
		Signal Response	
Before Injection	1	186.419	5.413
	2	39.000	2.739
	3	12.455	3.363
	4	13.667	3.230
	5	139.955	2.820
After Injection	6	86.818	21.973
	7	60.500	9.148
	8	48.143	4.486
	9	90.238	2.528
		SNRR	
Before Injection	1	82.213	42.117
	2	271.310	135.697
	3	34.489	29.015
	4	31.806	23.654
	5	1336.364	209.320
After Injection	6	11.383	8.863
	7	12.462	2.185
	8	1.943	.211
	9	4.748	1.147

cell. For each of these cells the mean and standard deviation of the signal response, noise response and SNRR were computed. Results are presented in Table 1. These measures were used to evaluate the nature of the sampling distribution of responses recorded under the experimental protocol of this experiment. In particular, they were used to ascertain the appropriateness of the application of parametric statistical methods to the data obtained in this manner (see discussion below). Note that this data is from a rat receiving the control solution only. The large differences apparent between the two Noise Response groups are most likely due to sampling bias arising from the use of multiple electrode penetrations to obtain the samples.

Figures 16 and 17 present two portions of the strip chart records obtained throughout the recording procedure for each rat. The top channel provides time calibration in 1-second intervals and also provides "tone-on" markers (heavy black bars below the time base line) as well as markers used to signal the drug injection duration and the periods of computer sampling (heavy black bars above the time base line). The second trace from the top is a record of the integrated spike count. The height of each peak is proportional to the number of spikes counted during that one-second interval. This trace provided an on line visual record of the noise response and the signal response. The third trace from the top presents the electro-

Figure 16

Strip Chart Record of Experimental Animal
at Time of 5-HTP Injection

(See text for explanation)

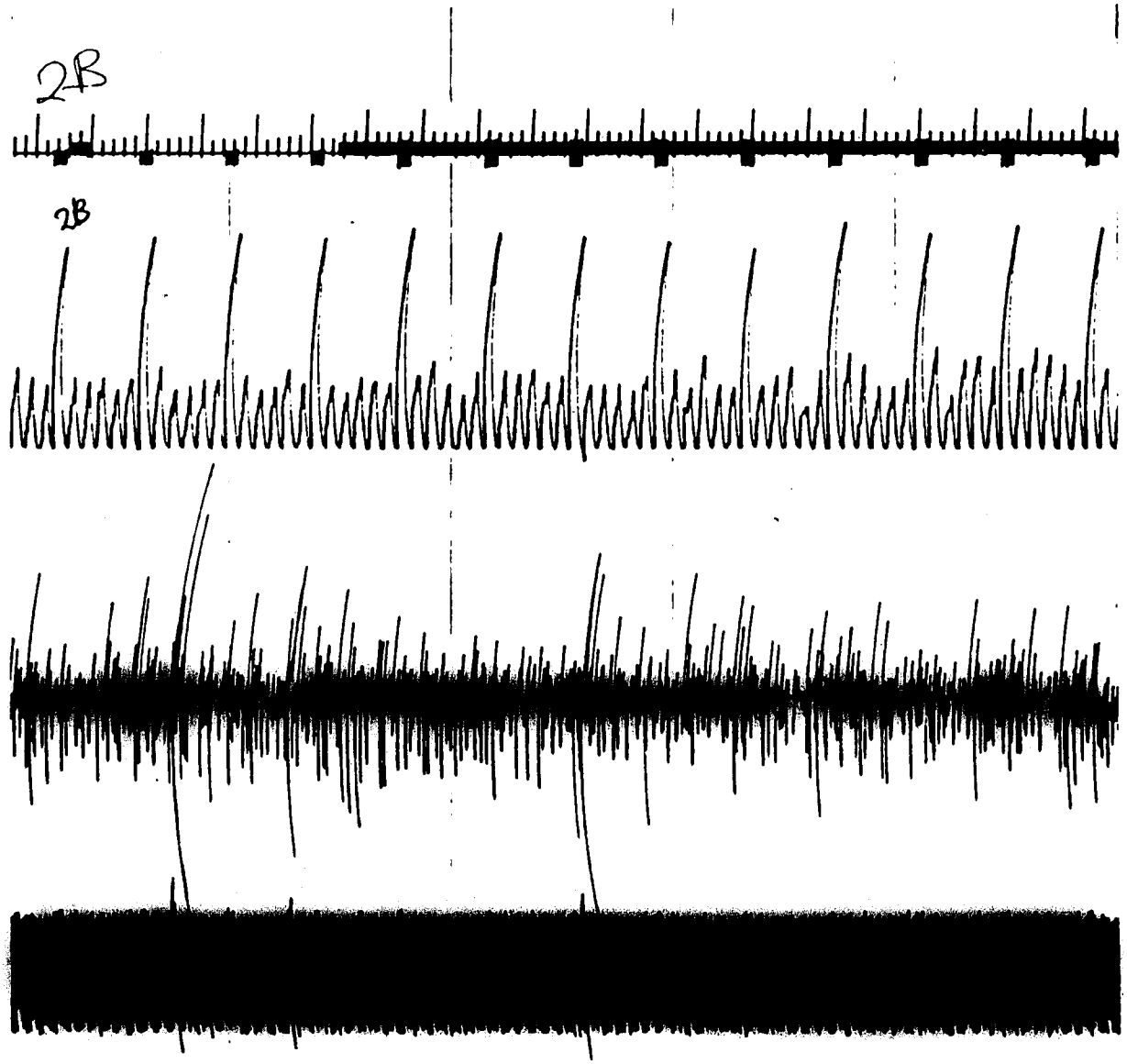
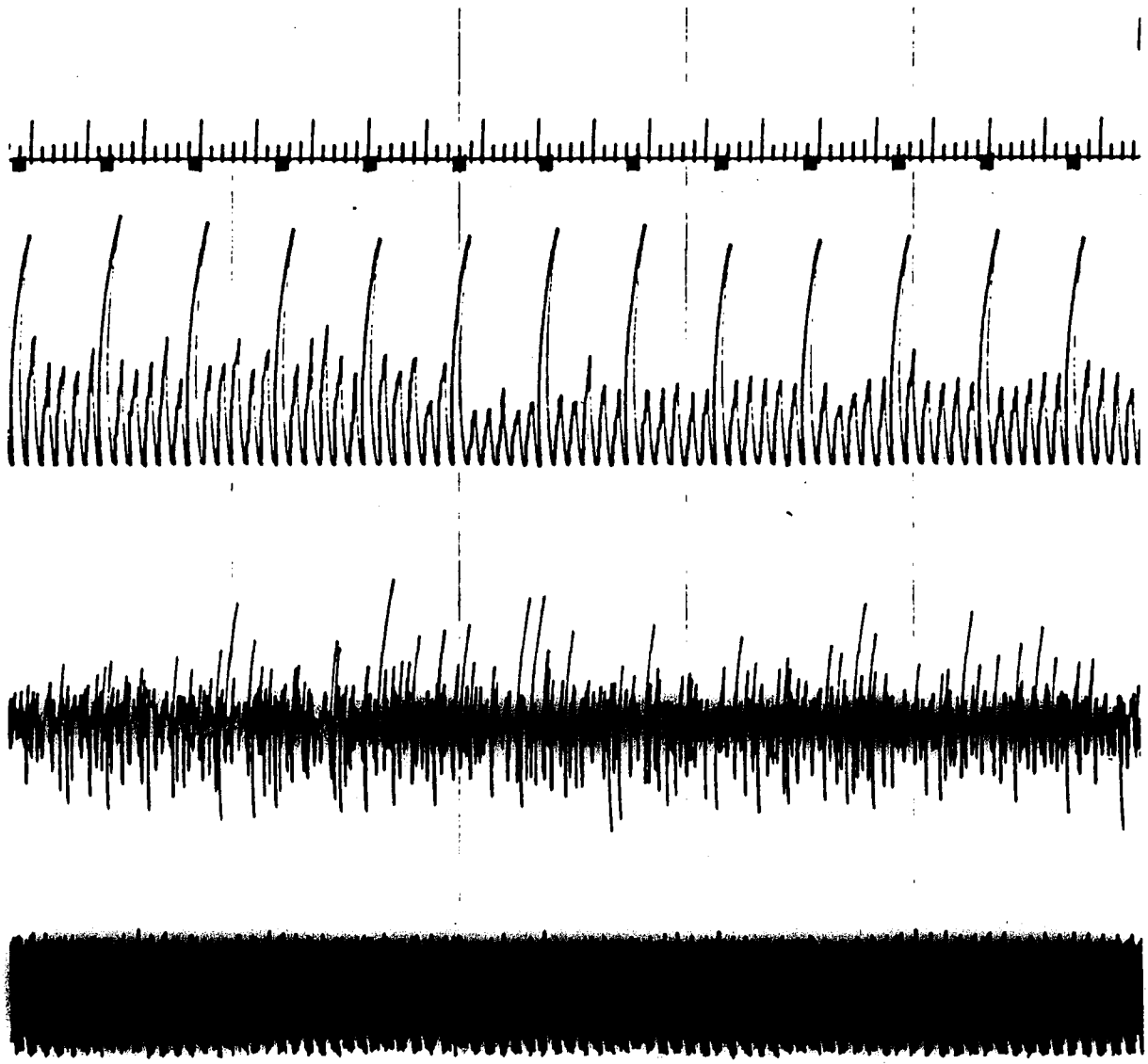

2B
2B

Figure 17

Strip Chart Record of Same Rat Shown in Figure 16 Taken One Hour Later

(See Text for Explanation)



encephalogram while the bottom trace is a combination of heart rate and respiration rate as recorded from the electrodes on either side of the chest. These bottom two channels were used to judge the physiological state of the animal and to provide a record of movement artifacts. Figure 16 shows the record of an experimental animal at the time of the 5-HTP injection. Figure 17 shows the record of the same rat approximately one hour later. A comparison of Figures 16 and 17 shows that the gross physiological state of the animal as indicated by these measures is relatively stable.

Discussion

There is little doubt from the evidence presented that all 18 cells included for discussion in this study were located in the cochlear nucleus (CN). All electrode penetrations entered the acoustic tubercle and the maximum depth of penetration (1.63 mm) is consistent with an electrode trajectory within the CN. Most convincing were the observations of a tonotopic arrangement of cells and complex frequency response characteristics. The observation of the "sandwich" type inhibitory and excitatory regions as seen by Møller (1969a) strongly indicate a CN origin, since a complex response characteristic of this type has been shown to be displayed by cells indigenous to this area.

The analysis of experimental data, particularly by means of statistical tests, necessitates a consideration of the applicability of the underlying assumptions of those tests. For parametric methods, and in particular the t test, it is assumed that the observations in question are selected from normally distributed populations which also have the same variance (Siegel, 1956). The validity of such assumptions with respect to the current research is in doubt. There are several population sources that might be of interest in research of this nature. One source of central importance to this paper is the single neuron. Presumably any single neuron could give rise to a population of noise response

values. Similarly it could engender populations of signal response values and of SNRR values. It must then be asked whether these populations are normal and whether any two populations, say the control and experimental populations, have the same variance. A failure to meet either of these conditions implies that, in this case, the use of a parametric statistical test is inappropriate. A perusal of Appendix A reveals that in a large number of cases the standard deviations of samples taken before and after the experimental injection are not similar. Using the Hartley test for homogeneity (Kirk, 1968) it was found that in 22 out of 27 cases the variances were significantly different ($p<.05$, $F(2,59)$). Although in some cases the sample number was larger than 60, even with this conservative estimate nearly all cases were shown to lack homogeneity. It may also be observed from Appendix A that the vast majority of difference scores for standard deviations show a larger variability after injection than before injection (i.e. negative difference scores). Based on these observations alone it would seem unwise to employ parametric methods, but in addition, the sample data reveal discrepancies from the normal distribution in terms of skew and kurtosis. (Kurtosis provides a measure of the "peakedness" of the distribution (Hays, 1973)). The values of skew and kurtosis for a normal distribution are 0.0 and 3.0 respectively. Of the sample values for skew, several were greater than 1.0, while the median value

was approximately 0.5. Kurtosis scores ranging as high as 116.1 and as low as 1.576 were obtained. Approximately half the scores were outside a range of 3.0 ± 0.6.

A second statistical source of interest was comprised of all the neurons of the cochlear nucleus. Theoretically this source could give rise to a population of the mean response values for each cell. Thus it might be desired to compare two samples of response values for the purpose of ascertaining if each sample arises from the same population before and after the experimental injection. Using values from Table 1, the standard deviations of the signal response means were calculated for the sample before injection (cells 1-5) and for the sample after injection (cells 6-9). The values obtained are $\sigma_1=71.48$ and $\sigma=17.70$ respectively. Performing similar calculations for the noise response means and SNRR means reveals the following results: Noise response - $\sigma_1=.94$, $\sigma_2=9.13$; SNRR - $\sigma_1=500.31$, $\sigma_2=4.42$. Again using the Hartley test, it was found that in all three cases the samples did not have similar variabilities ($p<.05$, $F(2,4)$). Consequently, the use of parametric methods for this case is also unwise. It should also be noted that, for example, in the case of the signal response, the sample means are 78.3 and 71.43 for the before and after injection samples. Comparing these values with the standard deviations obtained above, it appears that the variability of samples of this size can be quite

large. In view of the technical difficulty of obtaining samples of even this size, it appears that the sample variance cannot feasibly be reduced to a level which would allow the detection of small differences in the sample means.

This brings us to the third statistical source to be considered in this paper. We may conceive of two statistical populations which arise from measurements taken from two groups of rats, controls and experimentals. In this particular case the measurements are the difference scores shown in Appendix A. Performing calculations for the standard deviations and sample means as was done for the data of Table 1, the following results are obtained:

Signal Response:	σ_1=16.44, σ_2=6.54; $\bar{X}_1$=4.02, $\bar{X}_2$=5.80
Noise Response:	σ_1= 1.79, σ_2=1.37; $\bar{X}_1$=-.46, $\bar{X}_2$=-4.63
SNRR:	σ_1=38.94, σ_2=0.54; $\bar{X}_1$=-25.45, $\bar{X}_2$=0.02

The Hartley test revealed that, in the case of the SNRR the sample variabilities were significantly different ($p<.05$, $F(2,4)$). It is for this reason that the Mann-Whitney U test was chosen as the most appropriate method of analysis for this data. (The Mann-Whitney U test postulates only that the samples are independent and that the data may be assembled in a rank order (Siegel, 1956)). Furthermore, in the opinion of the

author, this experimental protocol is not able to engender sample data of sufficiently low variance to allow detection of any but the most robust of experimental effects.

As the statistical results indicate and as a visual inspection of Figures 10-15 corroborate, no consistent differences can be observed between those rats receiving 5-HTP and those rats receiving only the vehicle. Consequently the results of this experiment do not support the hypothesis that there exists a serotonergic input to the cochlear nucleus that acts to modulate the activity of the auditory pathways. These results may be a consequence of the inability of the experimental manipulations to show the activity of such a modulatory input or the consequence of the actual lack of such an input. As described earlier, there is ample evidence for the existence of non-afferent inputs to the cochlear nucleus and an indication that at least one of these efferent paths (the olivo-cochlear bundle) may be cholinergic in nature. Thus it may be that the modulatory inputs to the CN are not appreciably affected by alterations in brain 5-HT content. Alternatively, there may exist serotonergic inputs to the auditory pathways but not at the level of the CN. In this respect one is forced to wonder about the origin and function of the 5-HT terminals in the CN, inferior colliculus, and medial geniculate as observed by Fuxe (1965).

There are a variety of reasons to support the contention that the experimental manipulations of this study merely failed to detect the presence of the hypothesized input. The most obvious initial observation of the experimental protocol is that the data are being taken from a subject under highly artificial conditions. The surgical manipulations and anesthetic drugs are sure to have some effect on the normal functioning of the subject's nervous system. These effects can, at best, only be held to a minimum. In addition, the insertion of a microelectrode into the nervous tissue will disrupt not only those cells penetrated but also the interneuronal connections of the immediate area.

Secondly, it is not known for certain if the I.P. administration of 5-HTP actually affected the functionally important stores of 5-HT and it is not known if the pretreatment with PCPA afforded a depletion of serotonin that was functionally important. Thus it is possible that, with respect to the functionally important 5-HT pools, there was no difference after 5-HTP administration.

A third difficulty arises from time dimensional differences between the response measure and the experimental manipulations. On the one hand, single cell responses are measured on a scale of milliseconds. On the other hand the increase in 5-HT levels as a result of 5-HTP administration is measured in units four or five

orders of magnitude larger. To strike an analogy, the task is one of correlating a response measured in terms of minutes, say bar pressing, with an experimental manipulation measured in terms of years. Such relationships may be detected if the effect is large enough to be extracted from the variance of the response caused by the multitude of uncontrolled factors acting during the time course of the experimental manipulation. In the case of the current study this may not have been possible. In fact, as Møller (1969a) found, there do seem to be unexplained changes in spontaneous firing rates over periods of several minutes.

Another dimensional difficulty arises with the sampling method. Single unit techniques allow the experimenter to sample only a minute fraction of the cell population of most vertebrate nuclei. Usually it is implicitly assumed that the responses of one or a few neurons can provide information representative of the population of neurons. This assumption is manifested in the concept of "feature detector" neurons. As Erickson (1974) has pointed out, it is entirely possible that the relevant stimulus features are only unequivocally coded by the activity of the entire population and thus the importance of any one cell's activity becomes negligible. From this it seems possible that the effects of a modulatory circuit cannot be detected in the activity of any one cell or even from a group of cells unless it is known how those cells are interrelated. These considerations can account

for the positive results seen by Key (1965) with gross electrodes; results that remained undetected by the single unit methods of the present study. Key's results might also have been due to his use of LSD rather than 5-HTP, the former acting through some mechanism other than a serotonergic input to the CN.

A final source of difficulty may be the nature of the auditory stimulus. The use of a constantly repeating tone burst over the long recording period would be expected to cause habituation of the responses to successive bursts. This possibility was taken into account and all recordings were made only after a five minute habituation period. (In one case habituation was indeed observed informally by turning off the stimulus for a few minutes after the habituation period. When the stimulus was turned on again the response magnitude was noticeably greater.) It is possible that the hypothetical input may be active only in the unhabituated state and would thus be undetectable by the present techniques.

The use of a tone stimulus tuned to the characteristic frequency of each cell may not have been the most effective stimulus for uncovering the effects of a modulatory input. Greenwood and Maruyama (1965) discussed the advantages of using a tone plus white noise stimulus rather than white noise alone to study the interaction of excitatory and inhibitory inputs to a cell. Although this interaction seems to be of primary importance to the determi-

nation of a cell's responses, the present experiment examined only the sustained firing rate over a one second period. Obviously a variety of other measures, such as the temporal pattern of the one-second response, could be more sensitive to the actions of a modulatory input.

In the light of the foregoing comments, it is felt by the author that the importance of the research reported in this paper lies in the elucidation of the important experimental variables which must be taken into account in further studies of this type. The considerations of appropriate investigative and analytical techniques as provided above are particularly worthwhile in this respect.

Appendix A

Means and Standard Deviations of 9 Cells Recorded Before and After the Experimental Injection

Group	Cell No.	Mean Spike Rates		Standard Deviations	
		Before	After	Before	After
Noise Response					
Control					
	2/22	22.84	24.82	2.45	4.99
	3/12	.44	.17	.53	.26
	3/15	12.55	14.82	1.15	1.84
	3/20	6.19	4.06	2.16	1.33
Experimental					
	2/13	20.71	25.05	2.79	10.18
	3/01	15.21	22.30	1.09	3.06
	3/04	52.65	47.70	11.75	12.62
	3/08	99.51	96.26	3.86	7.55
	3/19	9.92	6.41	1.30	1.70
Signal Response					
Control					
	2/22	74.60	62.79	4.04	16.35
	3/12	53.64	50.55	3.27	4.29
	3/15	22.95	22.35	2.54	3.18
	3/20	23.35	54.92	4.53	9.73
Experimental					
	2/13	46.73	37.12	5.12	10.31
	3/01	44.39	51.17	3.15	2.60
	3/04	85.21	75.38	8.24	11.47
	3/08	231.92	221.34	4.98	15.63
	3/19	32.29	26.42	3.03	4.73
SNRR					
Control					
	2/22	3.29	2.56	.321	.783
	3/12	263.92	355.75	191.951	171.830
	3/15	1.84	1.54	.241	.327
	3/20	4.44	14.82	2.295	4.859
Experimental					
	2/13	2.28	1.91	.382	1.212
	3/01	2.94	2.34	.369	.354
	3/04	1.65	1.64	.190	.267
	3/08	2.33	2.31	.106	.206
	3/19	3.30	4.28	.460	.920

Appendix B

Skew and Kurtosis of 9 Cells Recorded Before and After the Experimental Injection

Group	Cell No.	Skew		Kurtosis	
		Before	After	Before	After
		Noise Response			
Control					
	2/22	-.288	-1.156	2.585	-4.372
	3/12	1.555	2.175	4.979	10.041
	3/15	.255	.130	3.289	2.586
	3/20	.642	-.245	3.552	2.036
Experimental					
	2/13	1.554	-.420	6.714	2.202
	3/01	-.782	-.300	3.560	2.376
	3/04	3.246	.419	22.843	2.926
	3/08	-.451	.169	2.453	2.908
	3/19	-.081	2.035	2.987	14.977
		Signal Response			
Control					
	2/22	-.535	-1.353	3.265	4.984
	3/12	-.078	-3.085	3.107	24.823
	3/15	-.015	-.047	2.427	2.657
	3/20	.923	-.687	2.911	2.408
Experimental					
	2/13	.067	-.066	2.361	3.032
	3/01	-.140	.057	2.756	3.246
	3/04	.884	.679	4.717	3.259
	3/08	-.154	-8.241	2.047	116.100
	3/19	.053	.622	2.551	4.099
		SNRR			
Control					
	2/22	.519	.463	2.987	5.689
	3/12	.378	-.416	1.604	1.576
	5/15	.170	.264	2.785	2.706
	3/20	1.024	2.029	3.251	9.607
Experimental					
	2/13	.392	1.416	3.775	5.295
	3/01	.685	.551	3.063	2.592
	3/04	-1.429	.950	8.421	3.793
	3/08	3.100	-3.425	2.964	47.744
	3/19	.338	.781	2.648	4.722

Appendix C

Difference Measures of Scores Reported in Appendicies A and B

Group	Cell No.	Mean	St. Dev.	Skew	Kurtosis
			Noise Response		
Control					
	2/22	-1.98	-2.54	-.868	-1.787
	3/12	.27	.27	-.620	-5.062
	3/15	-2.27	-.69	.125	.703
	3/20	2.13	.83	.887	1.516
Experimental					
	2/13	-4.34	-7.39	1.974	4.512
	3/01	-7.09	-1.97	-.482	1.184
	3/04	4.95	-.87	2.827	19.917
	3/08	3.25	-3.69	-.620	-.455
	3/19	3.51	-.40	-2.116	
			Signal Response		
Control					
	2/22	11.81	-12.31	-1.888	-1.719
	3/12	3.09	-1.02	-3.163	-21.716
	3/15	.60	-.64	-.062	-.230
	3/20	-31.57	-5.20	1.610	.503
Experimental					
	2/13	9.61	-5.19	.133	-.671
	3/01	-6.87	.55	-.197	-.490
	3/04	9.83	-3.23	.205	1.458
	3/08	10.58	-10.65	8.087	-114.053
	3/19	5.87	-1.70	-.569	-1.548
			SNRR		
Control					
	2/22	.73	-.43	.056	-2.702
	3/12	-92.46	20.12	.794	.028
	3/15	.30	-.09	-.094	.079
	3/20	-10.38	-2.56	-1.005	-6.356
Experimental					
	2/13	.37	-.88	-1.024	-1.520
	3/01	.60	.02	.134	.471
	3/04	.01	-.08	-2.379	4.619
	3/08	.02	-.10	6.525	-44.780
	3/19	-.98	-.46	-.443	-2.074

References

Aghajanian,G.K. LSD and CNS Transmission. Annual Review of Pharmacology, 1972,12,157.

Aghajanian,G.K., Graham,A.W., & Sheard,M.H. Serotonin-Containing Neurons in Brain: Depression of Firing by Monoamine Oxidase Inhibitors. Science, 1970,169(3950),1100-1102.

Aghajanian,G.K., & Haigler.H.J. Direct and Indirect Actions of LSD, Serotonin and Related Compounds on Serotonin-Containing Neurons. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Aghajanian,G.K., Haigler,H.J., & Bloom,F.E. Lysergic Acid Diethylamide and Serotonin: Direct Actions on Serotonin-Containing Neurons in Rat Brain. Life Sciences, 1972,II(1), 615-622.

Alexander,G.J., Kopeloff,L.M., & Alexander,R.B. Anticonvulsive Effect of p-Chlorophenylalanine in Audiosensitive Mice. Life Science, 1971,10(I),877-882.

Aprison,M.H., & Hingten,J.N. Serotonin and Behavior: A Brief Summary. Federation Proceedings, 1972,31(1),121-129.

Beckman,A.L., Satinoff,E. Arousal from Hibernation by Intra-Hypothalamic Injections of Biogenic Amines in Ground Squirrels. American Journal of Physiology, 1972,222(4),875-879.

Bennett,J.P., & Snyder,S.H. Stereospecific Binding of D-Lysergic Acid Diethylamide (LSD) to Brain Membranes; Relationship to Serotonin Receptors. Brain Research, 1975,94,523-544.

Berridge,M.J., Prince,W.T. The Nature of the Binding Between LSD and a 5-HT Receptor: A Possible Explanation for Hallucinogenic Activity. British Journal of Pharmacology, 1974,51,269-278.

Bliss,E.L. Effects of Behavioral Manipulations Upon Brain Serotonin and Dopamine. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Bliss,E.L., Ailion,J., & Zwanziger,J. Metabolism of Norepinephrine, Serotonin and Dopamine in Rat Brain with Stress. The Journal of Pharmacology and Experimental Therapeutics, 1968,164(1), 122-134.

Bloom,F.E., Hoffer,B.J. Nelson,C., sheu,Y-s., & Siggins,G.R. The Physiology and Pharmacology of Serotonin Mediated Synapses. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Boakes,R.J., Bradley,P.B., Briggs,I., & Dray,A. Antagonism of 5-Hydroxytryptamine by LSD25 in the Central Nervous System: A Possible Neuronal Basis for the Actions of LSD25. British Journal of Pharmacology, 1970,40,202.

Boggan,W.O. Serotonin and Convulsions. In J. Barchas & E. Usdin (Eds.) Serotonin and Behavior. New York: Academic Press, 1973.

Boggan,W.O., Freedman,D.X., Lovell,R.A., & Schlesinger,K. Studies in Audiogenic Seizure Susceptibility. Psychopharmacologia, 1971, 20,48-56.

Bradley,P.B., & Briggs,I. Actions of Serotonin and Related Substances on Single Neurons in the Brain. Advances in Biochemistry and Psychopharmacology, 1974,10,159-166.

Bradley,P.B., & Key,B.J. The Effects of Drugs on Arousal Responses Produced by Electrical Stimulation of the Reticular Formation of the Brain. Electroencephalography and Clinical Neurophysiology 1958,10,97-110.

Brody, J.F. Behavioral Effects of Serotonin Depletion and of p-Chlorophenylalanine (a Serotonin Depletor) in Rats. Psychopharmacologia, 1970, 17(1), 14-33.

Chase, T.N., & Murphy, D.L. Serotonin and Central Nervous System Functioning. Annual Review of Pharmacology, 1973, 13, 181-197.

Chase, T.N., & Kopin, I.J. Stimulus Induced Release of Substances from Olfactory Bulb Using the Push-Pull Cannula. Nature, 1968, 217, 466-467.

Conner, R.L., Stolk, J.M., Barchas, J.D., & Levine, S. PCPA and Habituation to Repetitive Auditory Startle Stimuli in Rats. Physiology and Behavior, 1970, 5(11), 1215-1219.

Conner, R.L., Stolk, J.M., Levine, S. Effects of PCPA on Fighting Behavior and Habituation of Startle Response in Rats. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Comis, S.D., & Guth, P.S. The Release of Acetylcholine from the Cochlear Nucleus Upon Stimulation of the Crossed Olivo-Cochlear Bundle. Neuropharmacology, 1974, 13, 633-641.

Corrodi, H., & Jonsson, G. The Formaldehyde Fluorescence Method for the Histochemical Demonstration of Biogenic Monoamines: A Review of the Methodology. Journal of Histochemistry and Cytochemistry, 1971, 15, 65.

Dahlström, J., Häggendal, J., & Atack, C. Localization and Transport of Serotonin. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Dahlström, A., & Fuxe, K. Evidence for the Existence of Monoamine-Containing Neurons in the CNS. ACTA Physiologica Scandinavica, 1964, 62(232).

DeYoe, E.A., & Foster, H. A Low Cost Neural Spike Window Discriminator with Digital Readout. Physiology and Behavior, 1976, 16(3), 371-373.

Desmedt, J.E. Neurophysiological Mechanisms Controlling Acoustic Input. In G.L. Rasmussen & W.F. Windle (Eds.), Neural Mechanisms of the Auditory and Vestibular Systems. Springfield, Ill.: Publ. Charles C. Thomas, 1960.

Erickson, R.P. Parallel "Population" Neural Coding in Feature Extraction. In F.O. Schmitt & F.G. Worden (Eds.), The Neurosciences: Third Study Program. Cambridge, Mass.: MIT Press, 1974.

Fernstrom, J.D., & Wurtman, R.J. Control of Brain 5-HT Content by Dietary Carbohydrates. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Fuxe, K. The Distribution of Monoamine Terminals in the Central Nervous System. ACTA Physiologica Scandinavica, 1965, 64 suppl. 247, 37-85.

Grahame-Smith, D.G. Studies In Vivo On the Relationship Between Brain Tryptophan, Brain 5-HT Synthesis and Hyperactivity in Rats Treated with a Monoamine Oxidase Inhibitor and L-Tryptophan. Journal of Neurochemistry, 1971, 18, 1053-1066.

Grahame-Smith, D.G. Does the Total Turnover of Brain 5-HT Reflect the Functional Activity of 5-HT in Brain? In J. Barchas and E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Greenwood, D., & Maruyama, N. Excitatory and Inhibitory Response Areas of Auditory Neurons in the Cochlear Nucleus. Journal of Neurophysiology, 1965, 28, 863-892.

Gromova, E.A. Role of Endogenous Neurotropic Substances in the Trophic Function of the Nervous System. Nerv. Trofika Fiziol. Patol., 1970, 30-48.

Harrison, J.M., & Feldman, M.L. Anatomical Aspects of the Cochlear Nucleus and Superior Olivary Complex. Contributions to Sensory Physiology, 1970, 4, 95-142.

Harvey, J.A., Heller, A., & Moore, R.Y. The Effects of Unilateral

and Bilateral Medial Forebrain Bundle Lesions on Brain Serotonin. Journal of Pharmacology and Experimental Therapeutics, 1963, 140, 103.

Harvey, J.A., & Lints, C.E. Lesions in the Medial Forebrain Bundle: Relationship Between Pain Sensitivity and Telencephalic Content of Serotonin. Journal of Comparative and Physiological Psychology, 1971, 74(1), 28-36.

Harvey, J.A., Schlosberg, A.J., & Yunger, L.M. Behavioral Correlates of Serotonin Depletion. Federation Proceedings, 1975, 34, 1796-1801.

Harvey, J.A., & Yunger, L.M. Relationship Between Telencephalic Content of Serotonin and Pain Sensitivity. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior, New York: Academic Press, 1973.

Hays, W.L. Moments of Distribution. Statistics for the Social Sciences (2nd Ed.). New York: Holt, Rinehart, and Winston, 1973.

Hole, K., Fuxe, K., & Jonssen, G. The Effect of 5,7-Dihydroxytryptamine Lesions of Ascending 5-Hydroxytryptamine Pathways on Pain Sensitivity and Motor Activity. Experimental Brain Research, 1975, 23,(S), 92.

Hubbard, J.E., & DiCarlo, V. Fluorescence Histochemistry of Monoamine-Containing Cell Bodies in the Brain Stem of the Squirrel Monkey (Saimiri Scibreus). Journal of Comparative Neurology, 1973, 147, 553-566.

Hutchins, D.A., & Rogers, K.J. Some Observations on the Circadian Rhythm of Locomotor Activity of Mice After Depletion of Cerebral Monoamines. Psychopharmacologia, 1973, 31(4), 343-348.

Jouvet, M. Neurophysiology of the States of Sleep. Physiological Reviews, 1967, 47(2), 117-177.

Jouvet, M. Serotonin and Sleep in the Cat. In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior, New York: Academic Press, 1973.

Jouvet, M. Monoaminergic Regulation of the Sleep-Waking Cycle in the Cat. In F. Schmitt & F. Worden (Eds.), The Neurosciences: Third Study Program. Cambridge, Mass.: MIT Press, 1974.

Kang, S., & Green, J.P. Steric and Electronic Relationships Among Some Hallucinogenic Compounds. Proceedings of the National Academy of Science, 1970, 67(1), 62-67.

Kay, R.H. The Physiology of Auditory Frequency Analysis. Progress in Biophysics and Molecular Biology, 1974, 28, 109-188.

Katsuki, Y., Sumi, T., Uchiyama, H., & Watanabe, T. Electric Responses of Auditory Neurons in Cat to Sound Stimulation. Journal of Neurophysiology, 1958, 21, 569-588.

Kawai,N. Release of 5-Hydroxytryptamine from Slices of Superior Colliculus by Optic Tract Stimulation. Neuropharmacology, 1970, 9,395-397.

Kellog,C. Serotonin Metabolism in the Brains of Mice Sensitive or Resistant to Audiogenic Seizures. Journal of Neurobiology,

1971,2(3),209-219.

Key,B.J. The Effects of Drugs on Discrimination and Sensory Generalization of Auditory Stimuli in Cats. Psychopharmacologia, 1961,2,352-363.

Key,B.J. Effects of Lysergic Acid Diethylamide on Potentials Evoked in the Specific Sensory Pathways. British Medical Bulletin, 1965,21(1),30-35.

Kirk,R.E., Experimental Design: Procedures for the Behavioral Sciences Belmont, California: Brooks/cole, 1968.

Klein,D.C. Circadian Rhythms in Indole Metabolism in the Rat Pineal Gland. In F. Schmitt & F. Worden (Eds.), The Neurosciences: Third Study Program. Cambridge, Mass: MIT Press, 1974.

Konig-Bersin,P., Waser,P.G., Langemann,H., & Lichtensteiger,W. Monoamines in the Brain Under the Influence of Muscimol and Ibotenic Acid, Two Psychoactive Principles of Amanita Muscaria. Psychopharmacologia, 1970,18,1-10.

Kostowski,W., Giacalone,E., Garattini,S., & Valzelli,L. Electrical Stimulation of Midbrain Raphe: Biochemical, Behavioral, and Bioelectric Effects. European Journal of Pharmacology, 1969, 7,170-175.

Maj, J., Borzecki, Z., Lastowski, Z., & Wieloz, M. Effect of Noise on 5-Hydroxytryptamine and 5 Hydroxy-indoleacetic Acid Levels in Rats. Medical Proceedings, 1967, 18(6), 590-598.

Mc Farlain, R.A., & Bloom, J.M. The Effects of Para-chlorophenyl-alanine on Brain Serotonin, Food Intake, and U-maze Behavior.

Psychopharmacologia, 1972, 27(1), 85-92.

Meyers, K.M., Dickson, W.M., & Schaub, R.G. Serotonin Involvement in a Motor Disorder of Scottish Terrier Dogs. Life Science, 1973, 13(9), 1261-1274.

Meyers, R.D. The Role of Hypothalamic Serotonin in Thermoregulation. In J. Barchas and E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Minckler, J. The Central Nervous System. In J. Minckler (Ed.), Introduction to Neuroscience. Saint Louis: C.V. Mosby Co., 1972.

Modigh, K. Central and Peripheral Effects of 5-HTP on Motor Activity in Mice. Psychopharmacologia, 1972, 23, 48-54.

Møller, A.R. Unit Responses in the Cochlear Nucleus of the Rat to Pure Tones. ACTA Physiologica Scandinavica, 1969, 75, 530-541.

Møller, A.R. Unit Responses in the Rat Cochlear Nucleus to Repetitive, Transient Sounds. ACTA Physiologica Scandinavica, 1969, 75, 542-551.

Møller, A.R. Unit Responses in the Rat Cochlear Nucleus to Tones of Rapidly Varying Frequency and Amplitude. ACTA Physiologica Scandinavica, 1971, 81, 540-556.

Møller, A.R. Coding of Amplitude and Frequency Modulated Sounds in the Cochlear Nucleus of the Rat. ACTA Physiologica Scandinavica, 1972, 86, 223-238.

Møller, A.R. Dynamic Properties of Excitation and Inhibition in the Cochlear Nucleus. ACTA Physiologica Scandinavica, 1975, 93, 442-454.

Morgan, W.M., Yndo, C.A., McFadin, L.S. Daily Rhythmic Changes in the Content of Serotonin and 5-Hydroxyindoleacetic Acid in the Cerebral Cortex of Mice. Life Sciences, 1974, 14, 329-338.

Osen, K.K. Cytoarchitecture of the Cochlear Nuclei in the Cat. Journal of Comparative Neurology, 1969, 136, 453-484.

Page, I.H., & Carlsson, A. Serotonin. In Handbook of Neurochemistry. New York: Plenum Press, 1970.

Parent, A., & Poirier, L.J. The Medial Forebrain Bundle (MFB) and Ascending Monoaminergic Pathways in the Cat. Canadian Journal of Physiology and Pharmacology, 1969, 47(9), 781-785.

Pepeu, G., Garau, L., & Molas, M.L. Does 5-Hydroxytryptamine Influence Cholinergic Mechanisms in the Central Nervous System? Advances in Biochemistry and Psychopharmacology, 1974, 10, 247-252.

Pfalz, R. Efferent Crossed Inhibition in the Ventral Cochlear Nuclei. In A.R. Møller (Ed.), Basic Mechanisms in Hearing. New York: Academic Press, 1973.

Phillis, J.W., Tebēcis, A.K., & York, D.H. The Inhibitory Action of Monoamines on Lateral Geniculate Neurons. Journal of Physiology, 1967, 190, 563-581.

Rake, A.V. Involvement of Biogenic Amines in Memory Formation: The C.N.S. Indole Amine Involvement. Psychopharmacologia, 1973, 29(2), 91-100.

Rasmussen, G.L. Efferent Fibers of the Cochlear Nerve and Cochlear Nucleus. In G.L. Rasmussen & W.F. Windle (Eds.), Neural Mechanisms of the Auditory and Vestibular Systems. Springfield, Ill.: Charles C. Thomas, 1960.

Rasmussen, G.L. Relative Population of Afferent and Efferent Synapses of the Cochlear Nucleus. Anatomical Records, 1966, 154, 406.

Rasmussen, G.L. Efferent Connections of the Cochlear Nucleus. In A.B. Graham (Ed.), Sensorineural Hearing Processes and Disorders. Boston: Little, Brown, and Co., 1967.

Rebentische, E. The Influence of Serotonin and LSD on the Photically Stimulated Activity of the Rat's Visual Cortex. Psychopharmacologia, 1968, 13, 106-117.

Rinkel, M., De Shon, H.J., Hyde, R.W., & Solomon, H.C. Experimentsl Schizophrenia - Like Symptoms. American Journal of Psychiatry, 1952, 108, 572-578.

Rose, J.E. Organization of Frequency Sensitive Neurons in the Cochlear Nucleus Complex of the Cat. In G.L. Rasmussen & W.F. Windle (Eds.), Neural Mechanisms of the Auditory and Vestibular Systems. Springfield, Ill.: Charles C. Thomas, 1960.

Rupert, A., Moushegian, G., & Galambos, R. Unit Responses to Sound

from Auditory Nerve of the Cat. Journal of Neurophysiology, 1963, 26, 449-465.

Sabelli, H.C., & Giardina, W.J. The Influence of Inhibitors of Amine Metabolism on the Effects of Serotonin and its Metabolites on Photic Evoked Potentials in Rabbits. Experientia, 1971, 27(1), 64-65.

Schlesinger, K., Boggan, W.O., & Freedman, P.X. Genetics of Audiogenic Seizures: III. Time Response Relationships Between Drug Administration and Seizure Susceptibility. Life Sciences, 1970, 9(1), 721-729.

Segal, M. Physiological and Pharmacological Evidence for a Serotonergic Projection to the Hippocampus. Brain Research, 1975, 94, 115-131.

Sheard, M.H., & Aghajanian, G.K. Neural Release of Serotonin: Interaction With Drugs. In A. Cerletti & F.J. Bove, The Present Status of Psychotropic Drugs. Amsterdam: Excerpta Medical Foundation, 1969.

Shepherd, G.M. Neocortex. In The Synaptic Organization of the Brain. New York: Oxford University Press, 1974.

Siegel, S. Choosing an Appropriate Statistical Test. In Nonparametric Statistics for the Behavioral Sciences. New York: McGraw-Hill, 1956.

Spafford, D.C., & Pengelley, E.T. The Influence of Neurohumor Serotonin On Hibernation in the Golden-Mantled Ground Squirrel,

Citellus Lateralis. Comparative Biochemistry and Physiology, 1971, 38(2A), 239-250.

Tagliamonte, A., & Tagliamonte, P. The Effects of Psychotropic Drugs on Tryptophan Concentration in the Rat Brain. Journal of Pharmacology and Experimental Therapeutics, 1971, 177(3), 475-480.

Takagi, H., Satoh, M., Yamatsu, K., Kimura, K., & Nakama, M. Central Effects of 3,4-Dihydroxyphenylalanine and 5-Hydroxytryptophan on Tetrabenazine Pretreated Rabbits with Special Reference to the Possible Role of Catecholamine and Serotonin in the Brain. International Journal of Neuropharmacology, 1968, 7, 263-273.

Talarmain, Y., Valzelli, L., & Lehmann, A. Turn-over de la Serotonine. Ses Rapports Avec la Crise Audiogene de la Souris. Complets Rendus de la Societe de Biologie, 1970, 164, 52-56.

Tebecis, A.K. Are 5-Hydroxytryptamine and Noreadrenaline Inhibitory Transmitters in the Medial Geniculate Nucleus? Brain Research, 1967, 6, 780-782.

Thornton, A.R.D., & Coleman, M.J. The Adaptation of Cochlear and Brainstem Auditory Evoked Potentials in Humans. Electroencephalography and Clinical Neurophysiology, 1975, 39, 399-406.

Thurlow, W.R. Audition. In J.W. Kling & L.A. Riggs, Woodworth and Schlosberg's Experimental Psychology: Third Edition. New York: Holt, Rinehart, and Winston, 1971.

Tilson, H.A., & Sparber, S.B. Studies on the Concurrent Behavior and Neurochemical Effects of Psychoactive Drugs Using the Push-Pull

Cannula. Journal of Pharmacology and Experimental Therapeutics, 1972, 181, 387-398.

Van Gisbergen, J.A.M., Grashuis, J.L. Johannesna, P.I.M., & Vendrik, A.J.H. Spectral and Temporal Characteristics of Activation and Suppression of Units in the Cochlear Nuclei of the Anesthetized Cat. Experimental Brain Research, 1975, 23, 367-386.

Vermes, I., & Telegdy, G. Effects of Stress on Serotonin Metabolism in the Limbic System in Rats. Experimental Brain Research, 1975, 23(S), 208.

Von Bekesy, G. Current Status of Theories of Hearing. Science, 1956, 123, 779-783.

Wada, J., & Ikeda, H. Phenomenological and Pharmacological Identity of Audiogenic-Sensitive States in Animals Treated with Methionine Sulfoximine and in Genetically Audiogenic-Seizure-Susceptible Animals. Recent Advances in Biological Psychiatry, 1966, 9, 131-153.

Weissman, A. Behavioral Pharmacology of p-Chlorophenylalanine (PCPA). In J. Barchas & E. Usdin (Eds.), Serotonin and Behavior. New York: Academic Press, 1973.

Wurtman, R.J., & Fernstrom, J.P. Control of Brain Serotonin by the Diet. Advances in Neurology, 1974, 5, 19-29.

Yamanioto, C. Electrical Activity Recorded from Thin Sections of the Lateral Geniculate Body and the Effects of 5-Hydroxy-tryptamine. Experimental Brain Research, 1974, 19, 271-281.

Yoo, C.S., Lee, K.D., & Yoo, S.B. Change of Brain Serotonin Content by Thiopental. (Abstr.) Taehan Naekwahak Hoe Chapchi, 1968, 11(3), 179-182.

Vita

Edgar Ackerman DeYoe III was born in Paterson, New Jersey on February 11, 1950. He moved to the town of Whitney Point in upstate New York at the age of four and graduated from the Whitney Point Central School in 1968. He entered Lehigh University in the fall of that year, graduating in 1972 with a Bachelor of Science degree in Electrical Engineering. During his undergraduate career he was elected to the electrical engineering honor society, Eta Kappa Nu, as well as the national engineering honor society, Tau Beta Pi. He was included in the Dean's List for eight consecutive semesters, graduating with a 3.5 cumulative average. He took part in the French Summer Study Abroad program and a work-study cooperative program with I.B.M. Corporation.

After a two year leave, he entered the graduate program in Psychology at Lehigh University to study Physiological Psychology. He has been involved in several research projects and has published a short paper entitled "A Low Cost Neural Spike Window Discriminator with Digital Readout" in Physiology and Behavior (16(3), 1976, 371-373).